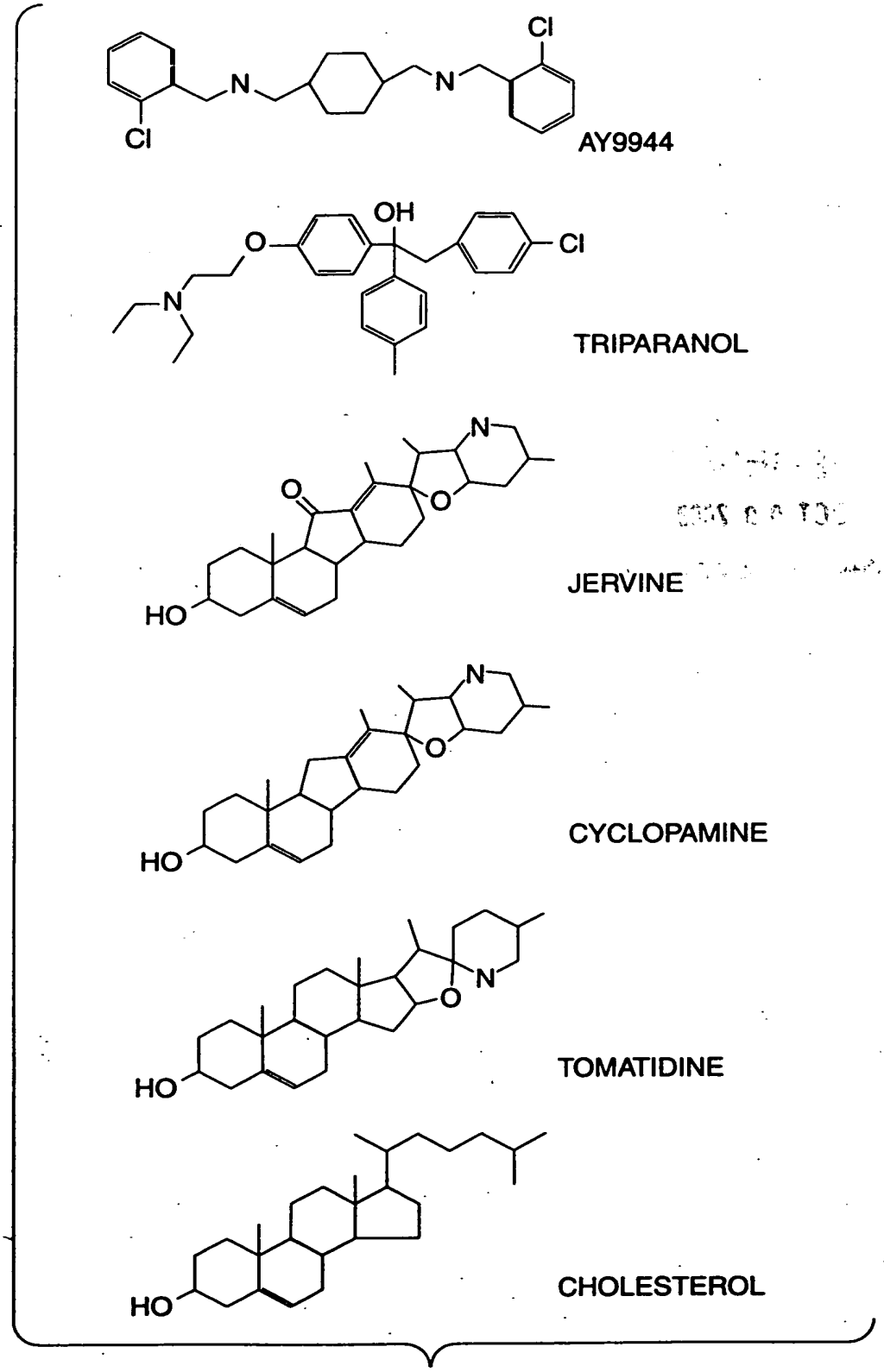

Cl
N
N
Cl
AY9944
OH
O
Cl
N
TRIPARANOL
N
O
O
HO
JERVINE
N
O
HO
CYCLOPAMINE
N
O
HO
TOMATIDINE
HO
CHOLESTEROL

Fig. 1

COMPOUND A

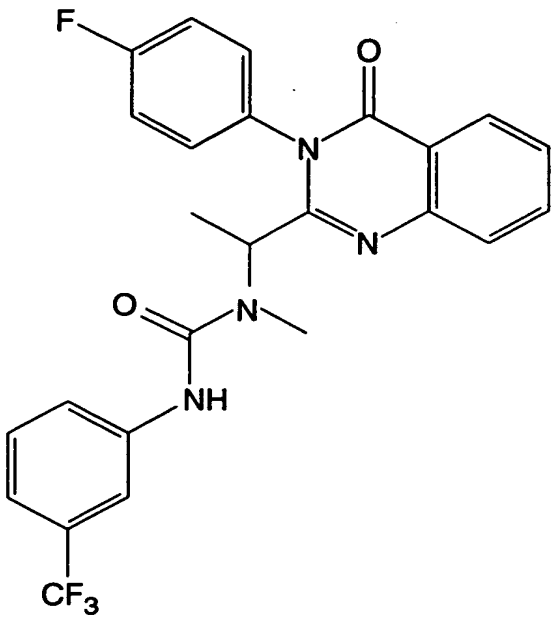


Fig. 2A

COMPOUND B

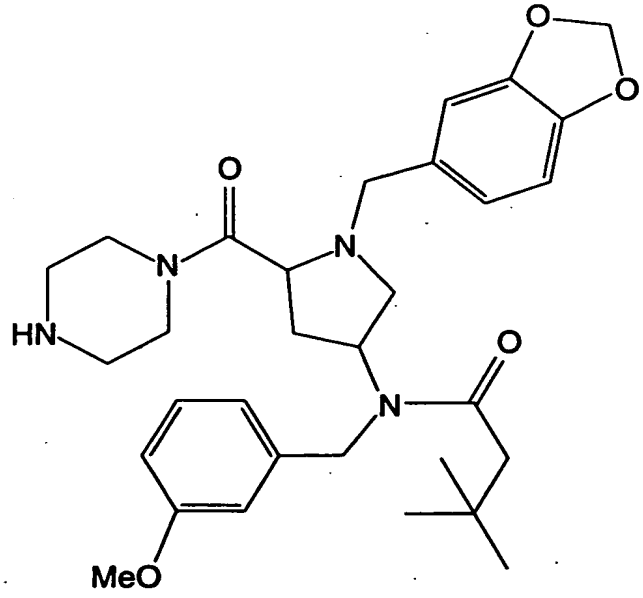
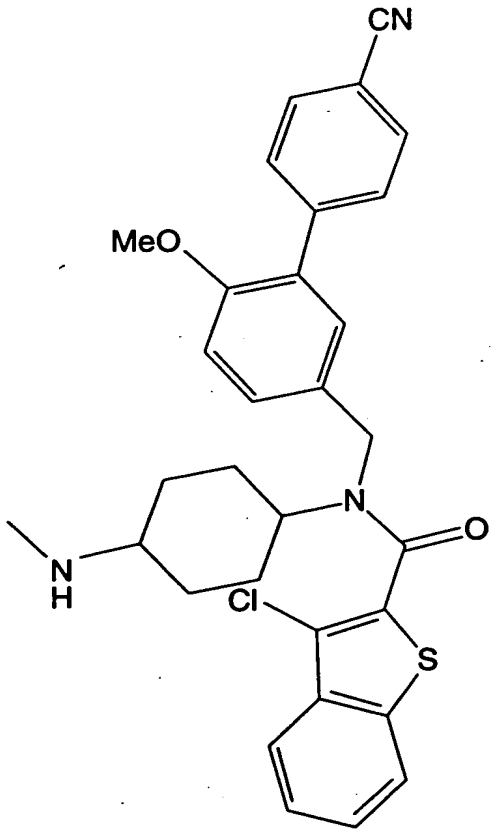


Fig. 2B



AGONIST Z

Fig. 3

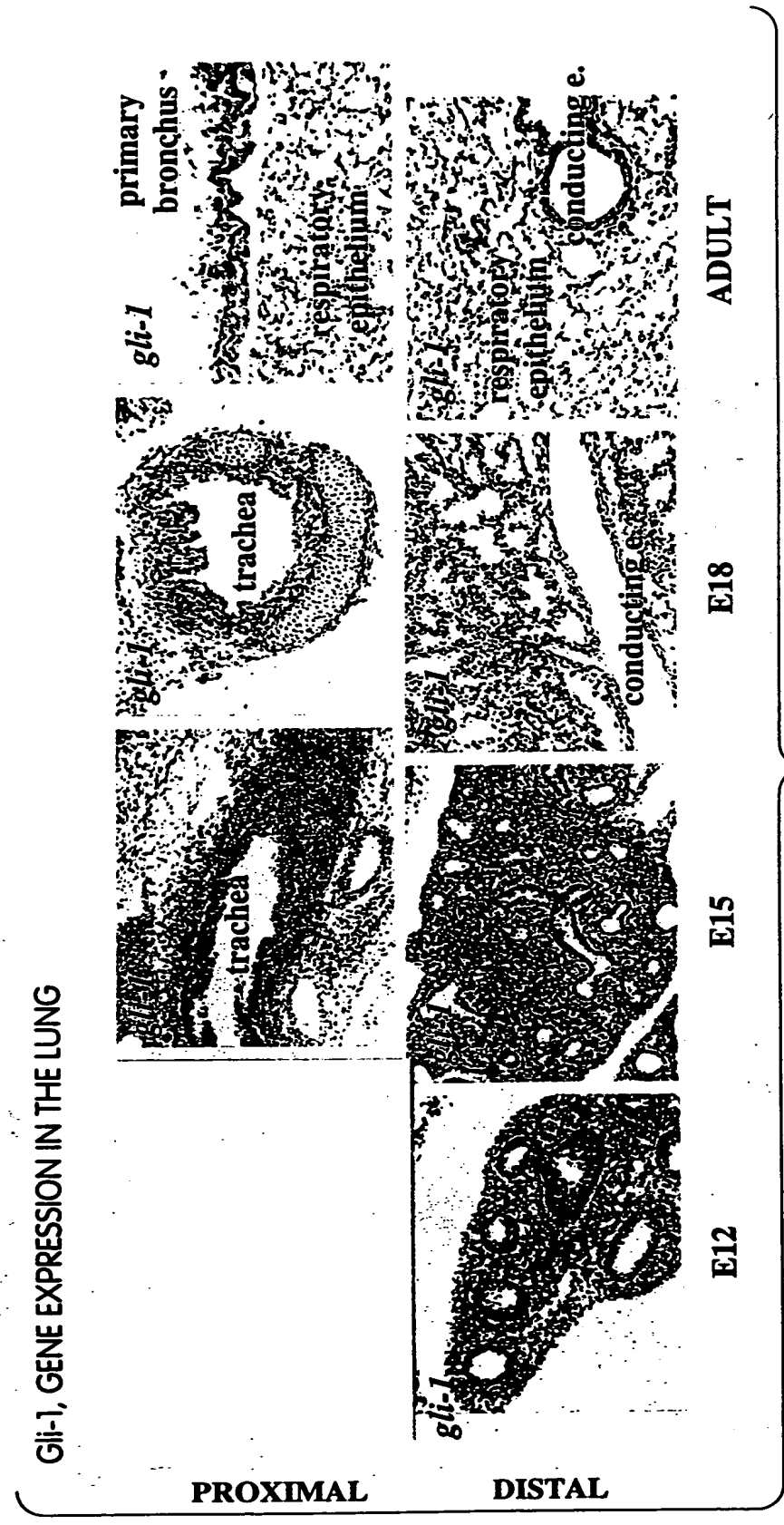


Fig. 4

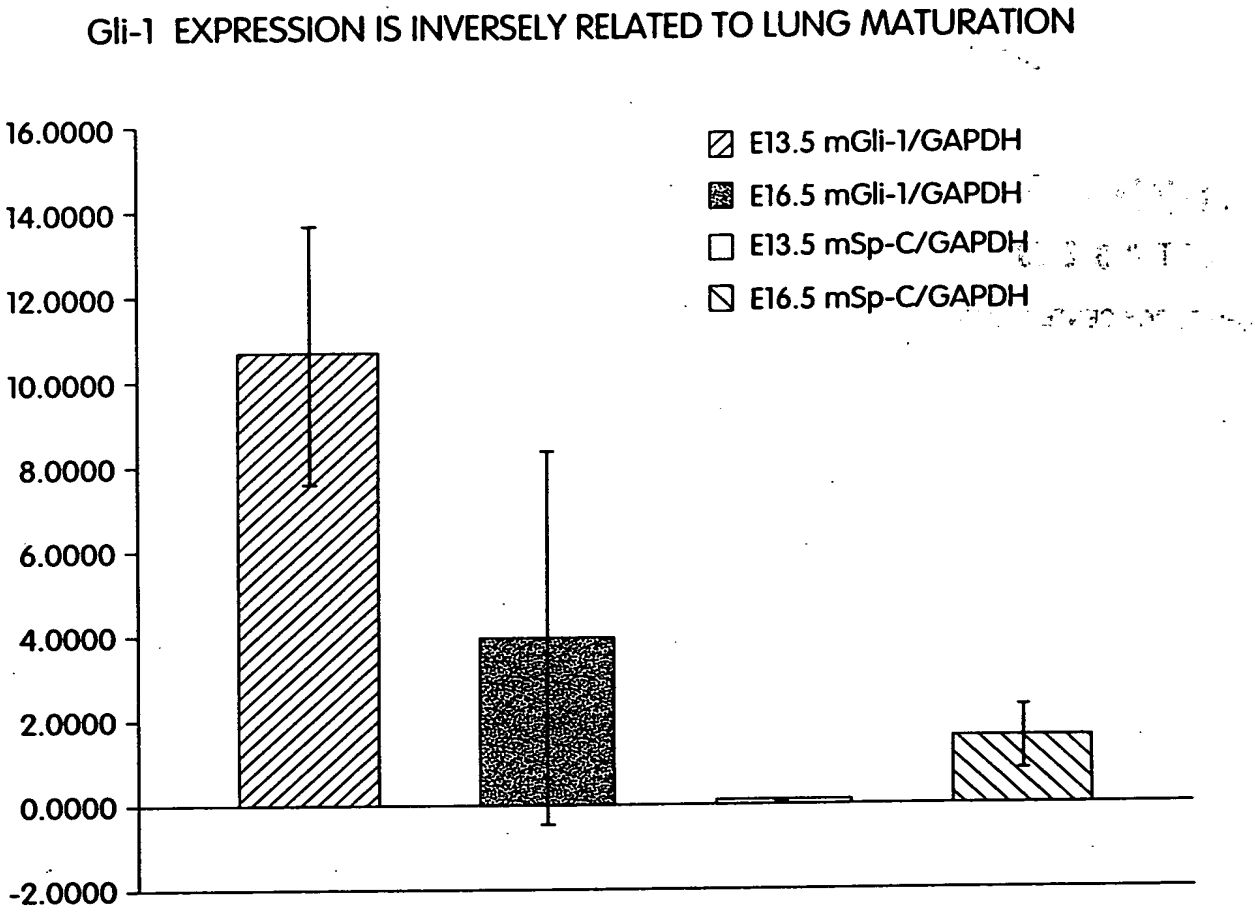

Gli-1 EXPRESSION IS INVERSELY RELATED TO LUNG MATURATION
16.0000
14.0000
12.0000
10.0000
8.0000
6.0000
4.0000
2.0000
0.0000
-2.0000
E13.5 mGli-1/GAPDH
E16.5 mGli-1/GAPDH
E13.5 mSp-C/GAPDH
E16.5 mSp-C/GAPDH

Fig. 5

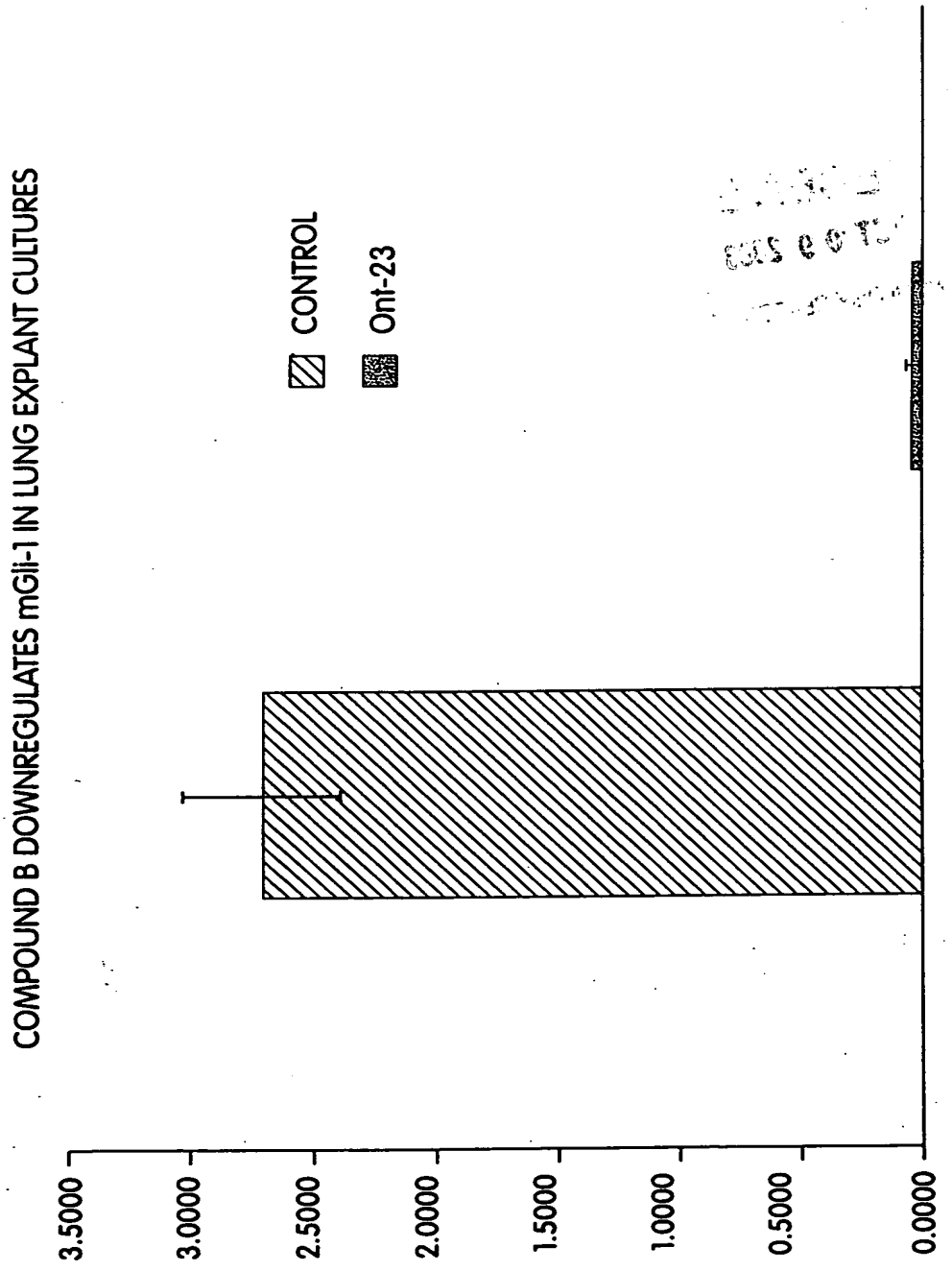

COMPOUND B DOWNREGULATES mGli-1 IN LUNG EXPLANT CULTURES
CONTROL
Ont-23
3.5000
3.0000
2.5000
2.0000
1.5000
1.0000
0.5000
0.0000

Fig. 6

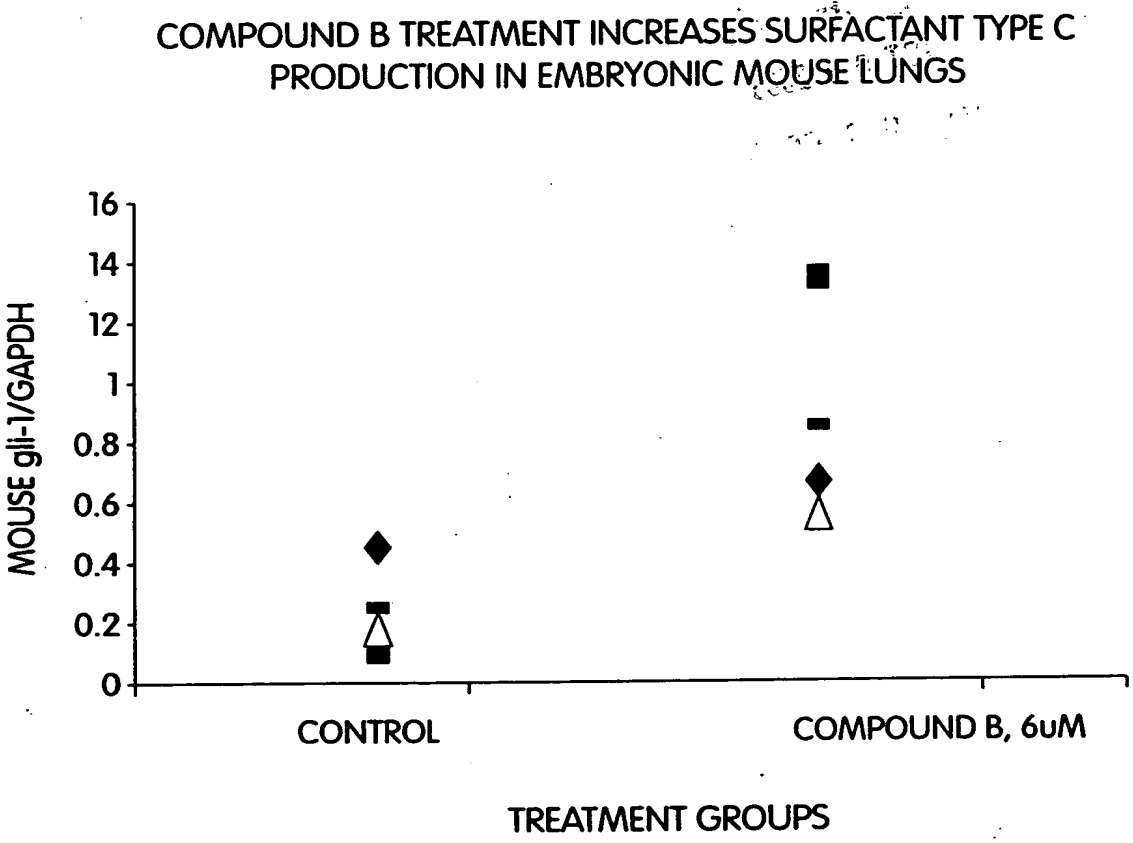

COMPOUND B TREATMENT INCREASES SURFACTANT TYPE C PRODUCTION IN EMBRYONIC MOUSE LUNGS
MOUSE gli-1/GAPDH
16
14
12
1
0.8
0.6
0.4
0.2
0
CONTROL
COMPOUND B, 6uM
TREATMENT GROUPS

Fig. 7

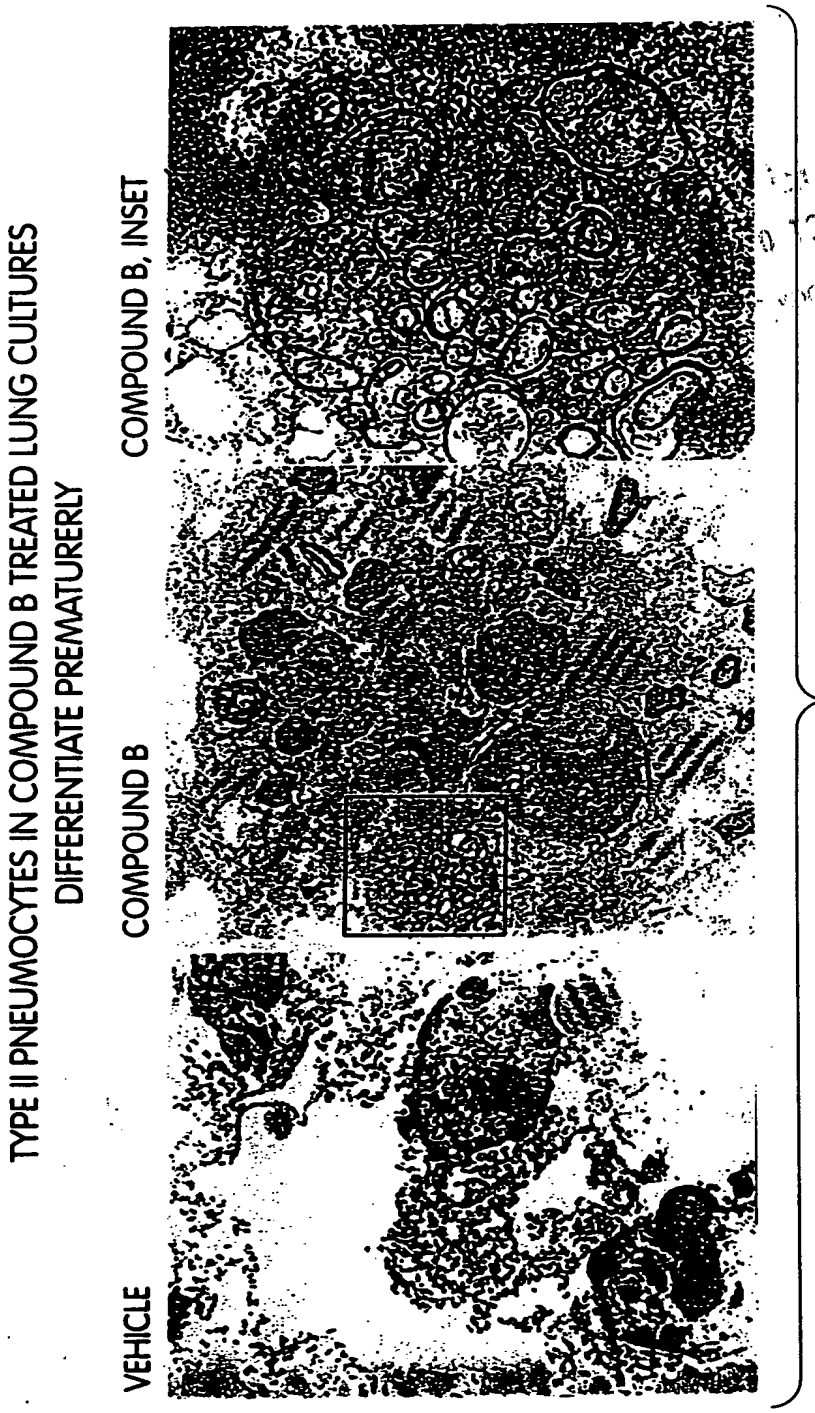


Fig. 8

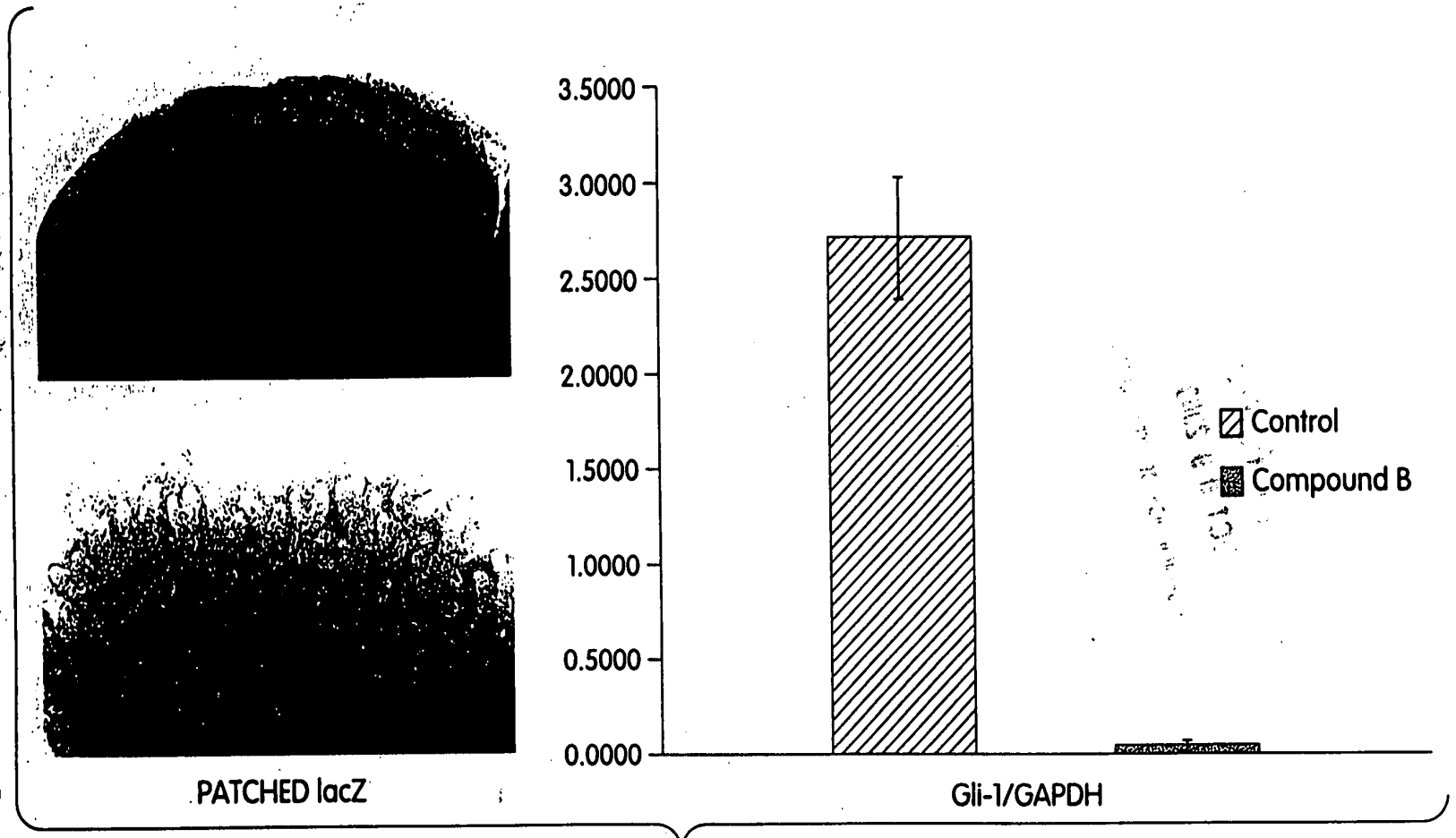

3.5000
3.0000
2.5000
2.0000
1.5000
1.0000
0.5000
0.0000
Control
Compound B
PATCHED lacZ
Gli-1/GAPDH

Fig. 9

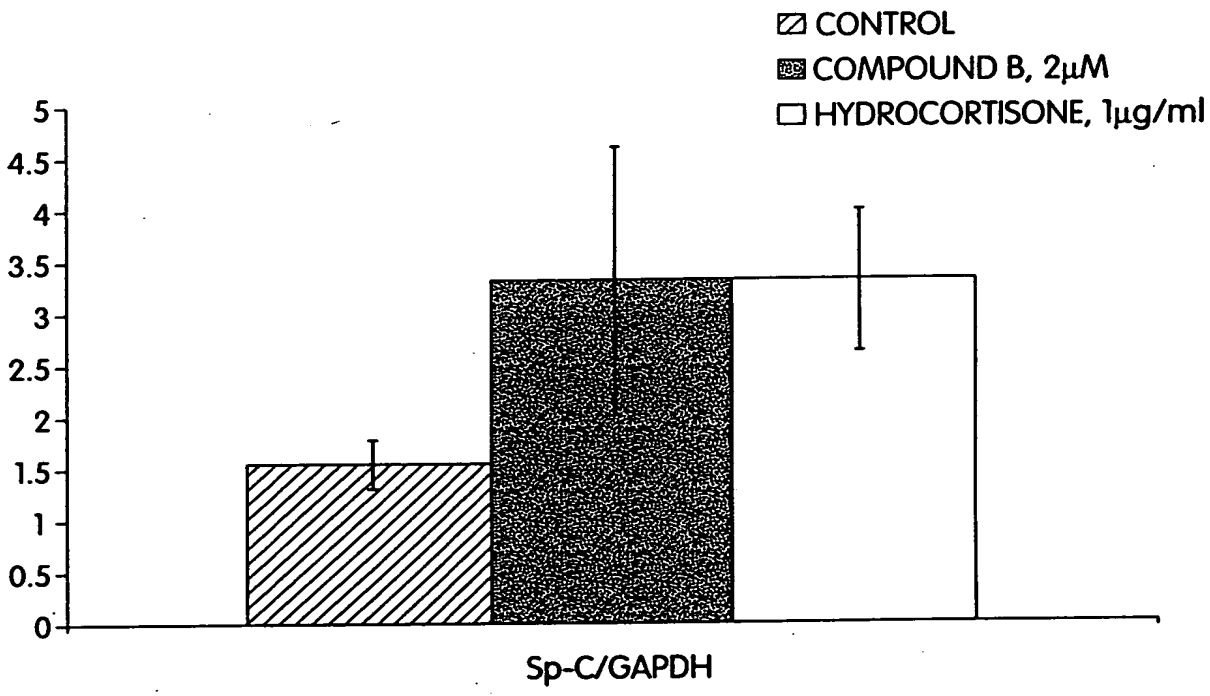

CONTROL
COMPOUND B, 2μM
HYDROCORTISONE, 1μg/ml
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Sp-C/GAPDH

Fig. 10

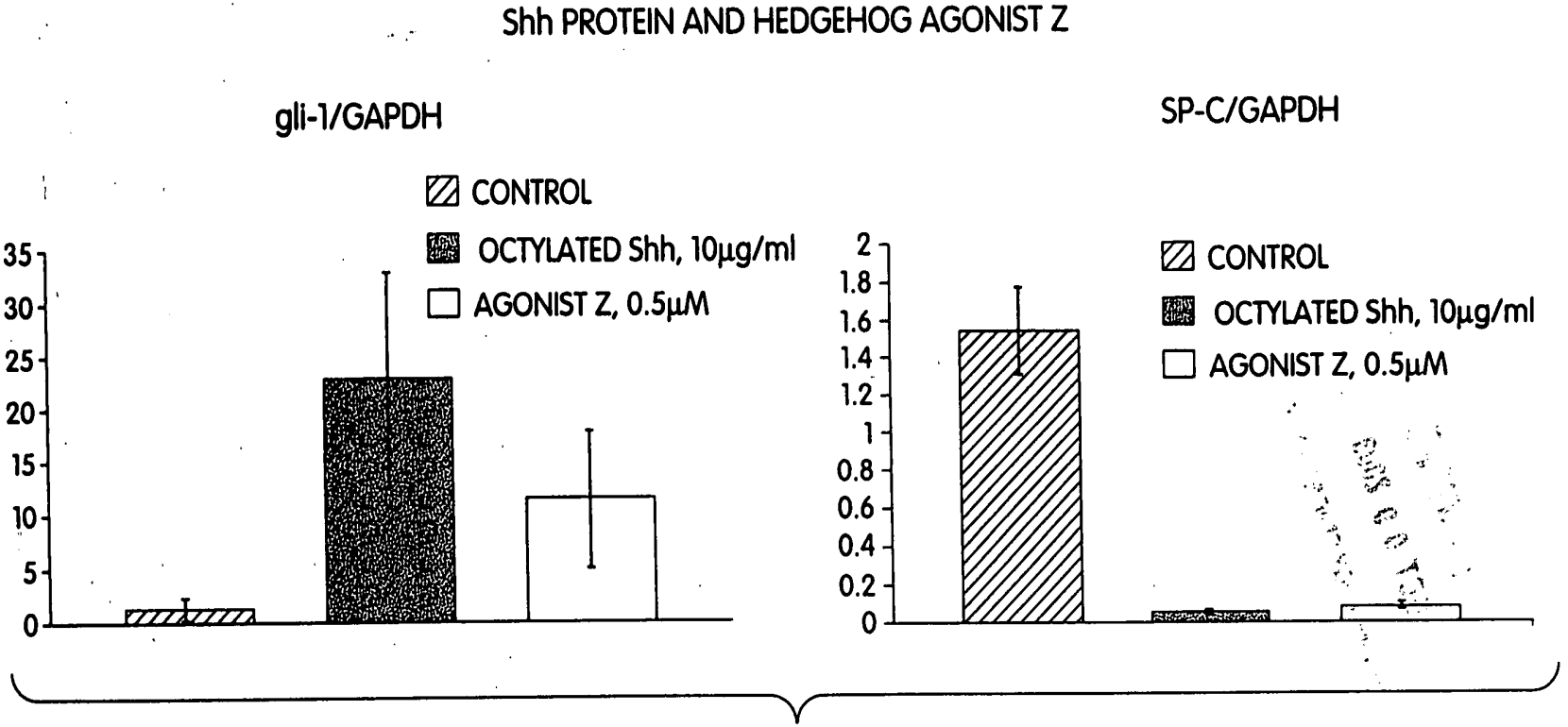

Shh PROTEIN AND HEDGEHOG AGONIST Z
gli-1/GAPDH
CONTROL
OCTYLATED Shh, 10µg/ml
AGONIST Z, 0.5µM
35
30
25
20
15
10
5
0
SP-C/GAPDH
CONTROL
OCTYLATED Shh, 10µg/ml
AGONIST Z, 0.5µM
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0

Fig. 11

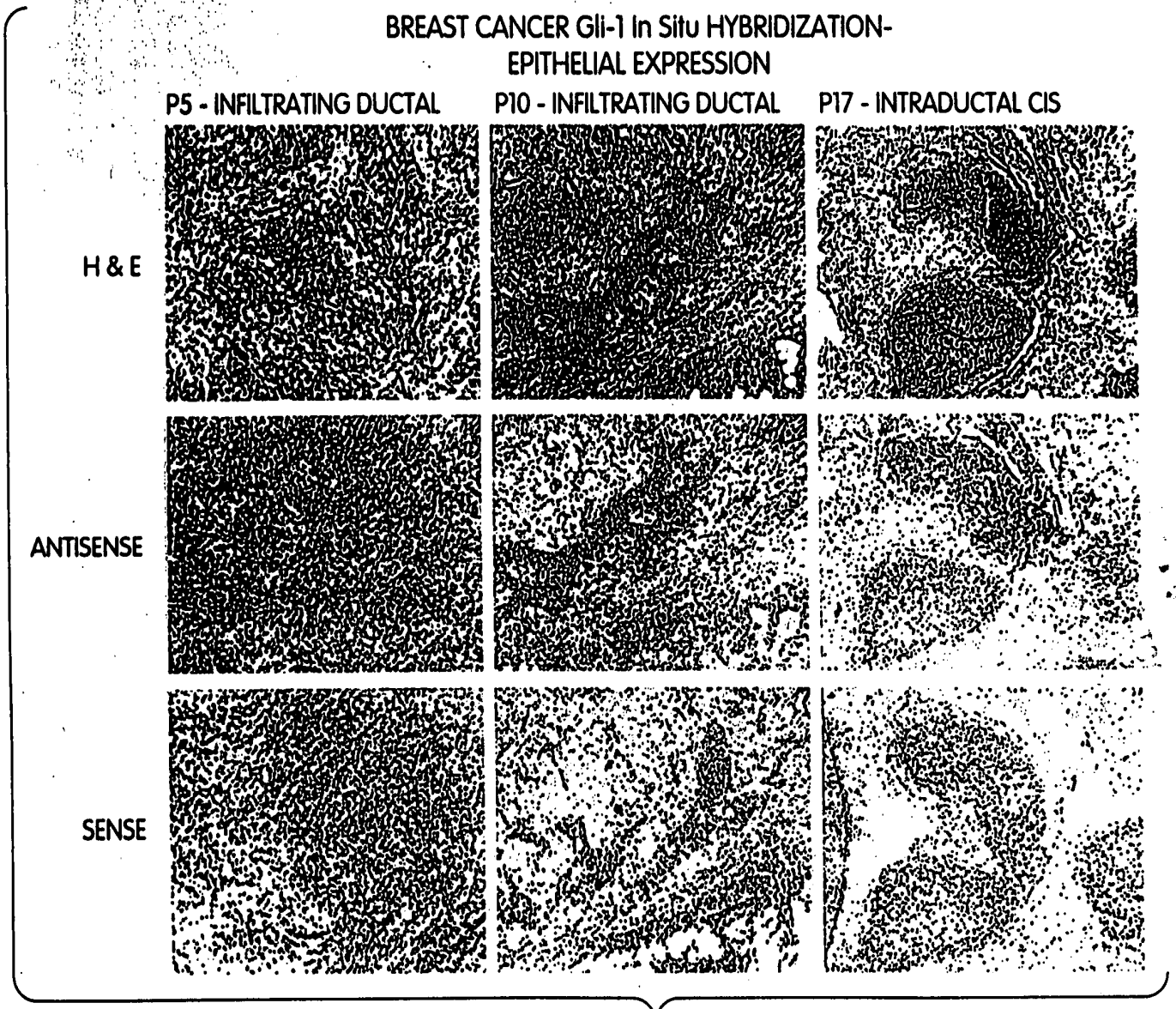


Fig. 12

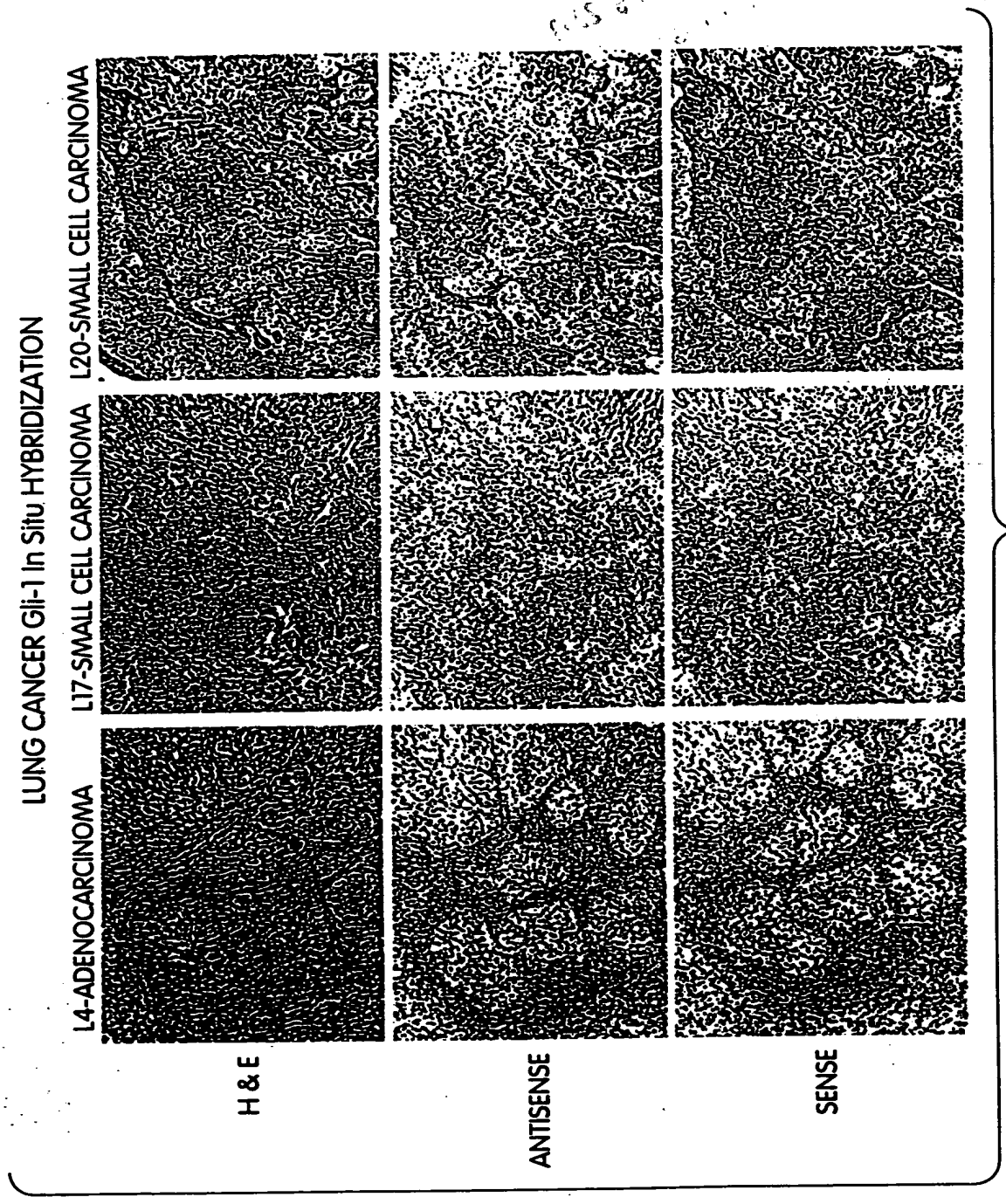


Fig. 13

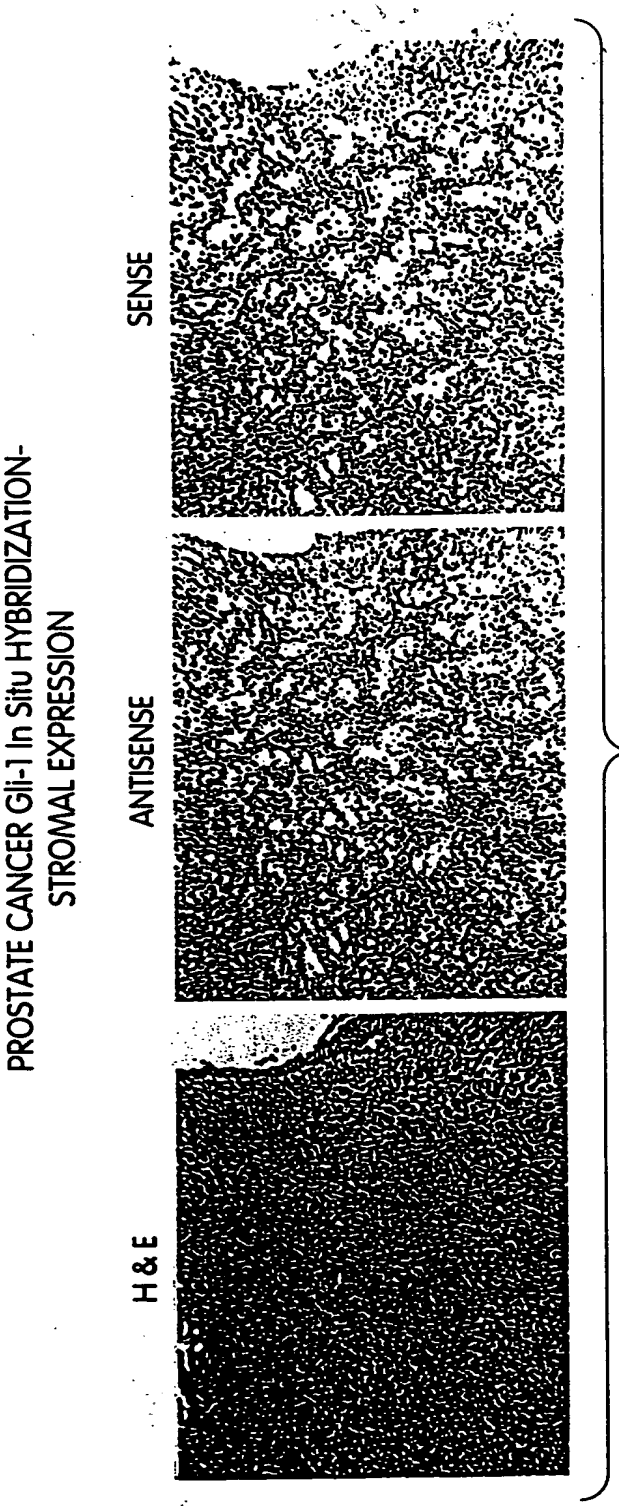


Fig. 14

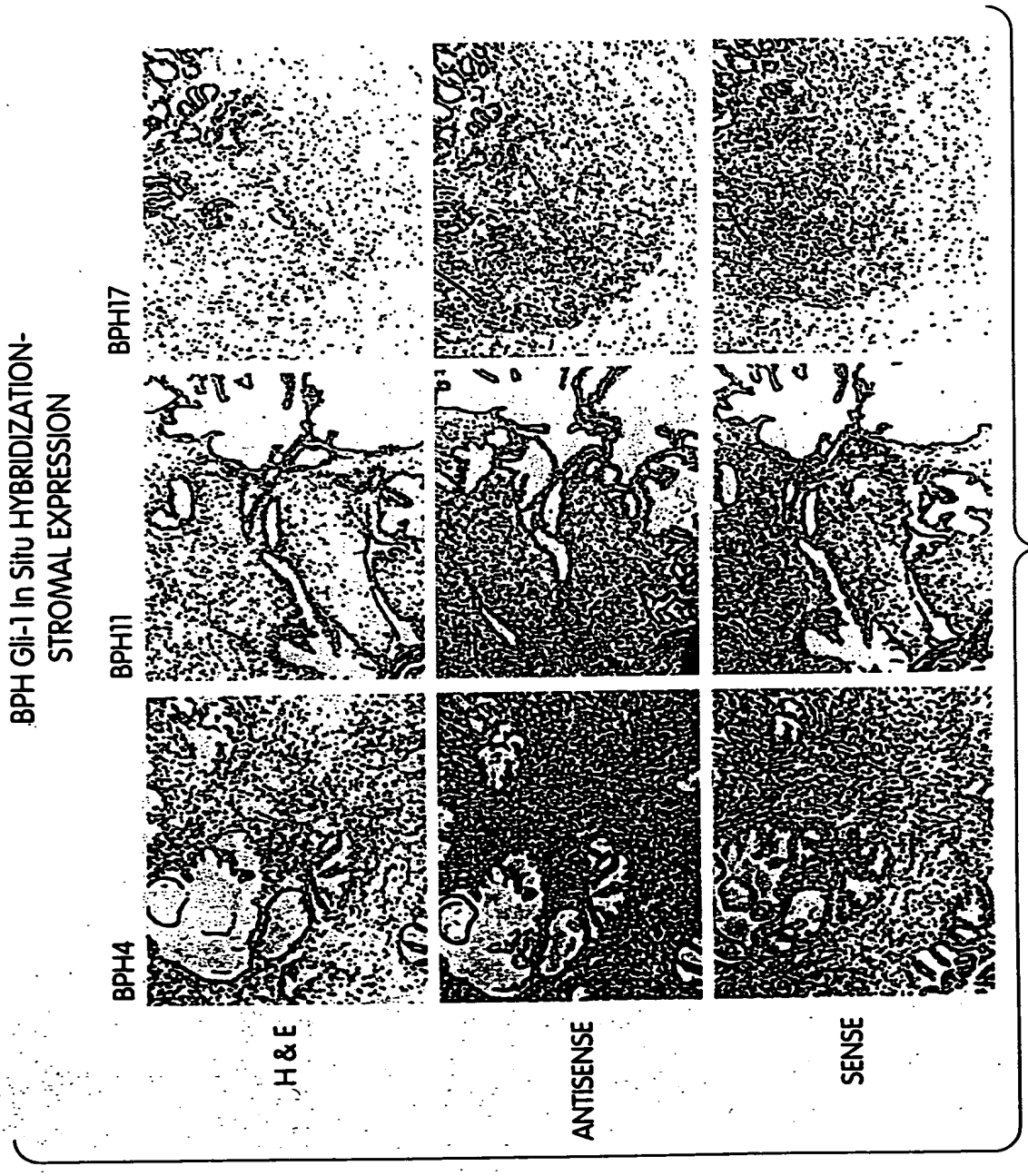


Fig. 15

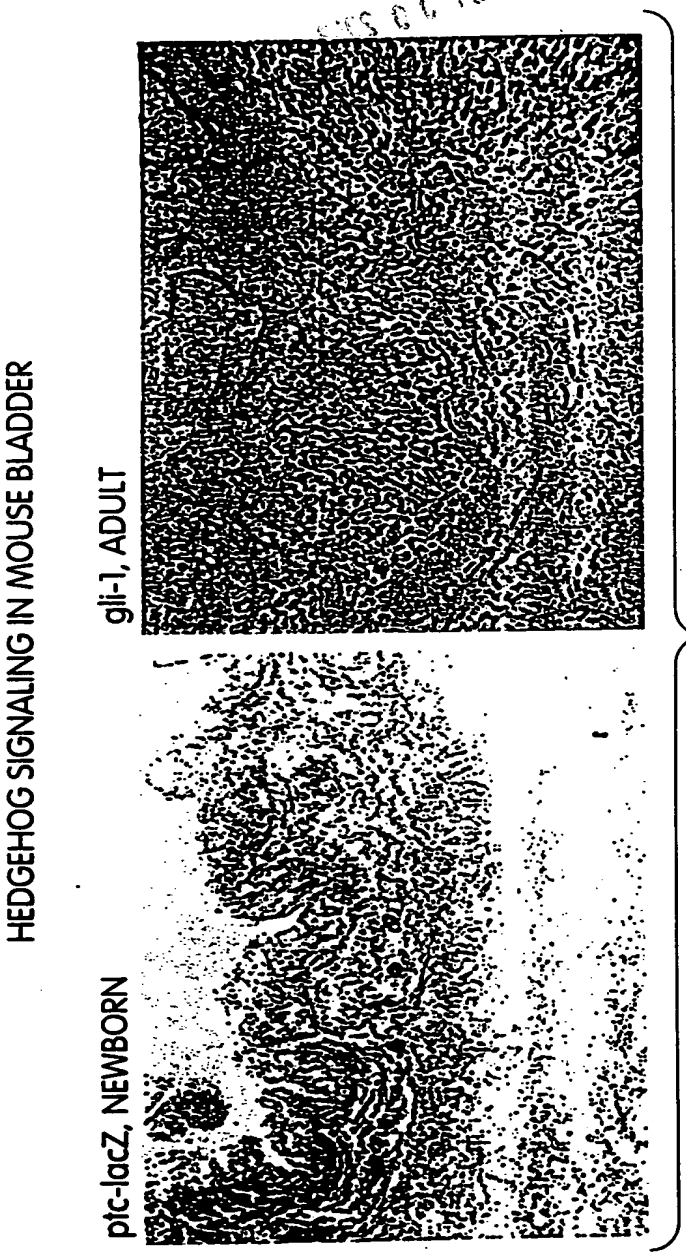


Fig. 16

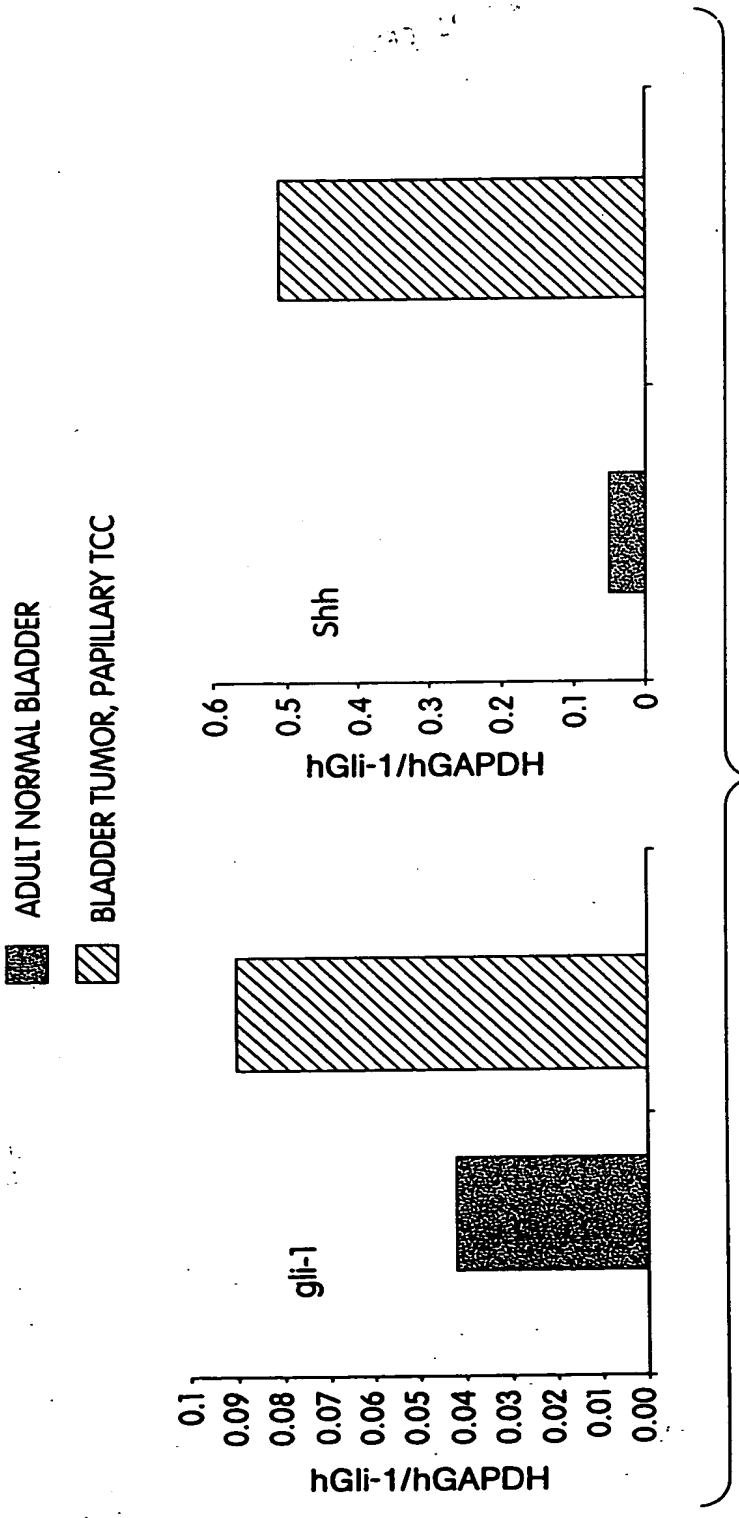

ADULT NORMAL BLADDER
BLADDER TUMOR, PAPILLARY TCC
gli-1
hGli-1/hGAPDH
0.1
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
Shh
hGli-1/hGAPDH
0.6
0.5
0.4
0.3
0.2
0.1
0

Fig. 17

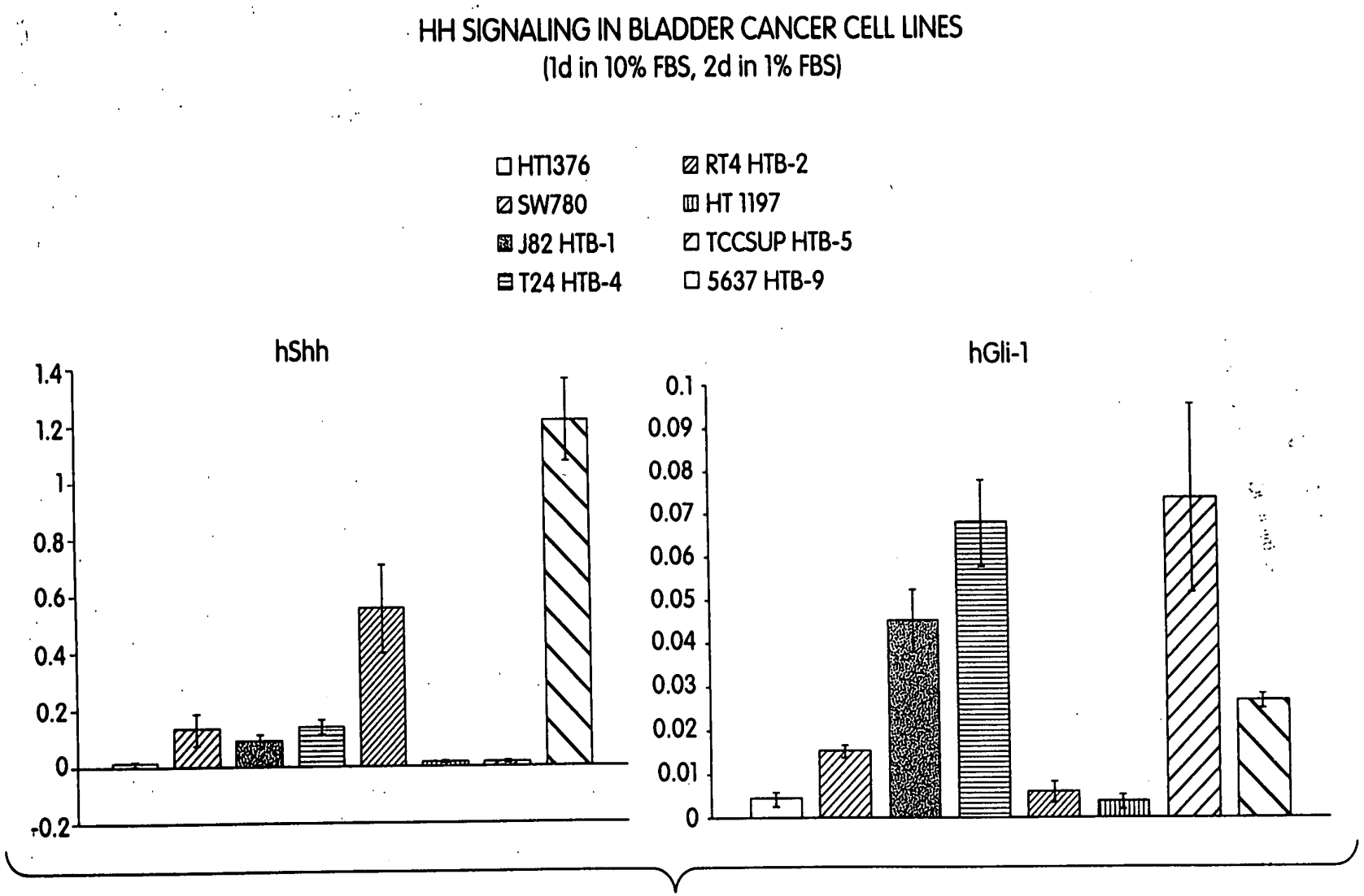

HH SIGNALING IN BLADDER CANCER CELL LINES
(1d in 10% FBS, 2d in 1% FBS)
HT1376
SW780
J82 HTB-1
T24 HTB-4
RT4 HTB-2
HT 1197
TCCSUP HTB-5
5637 HTB-9
hShh
1.4
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
hGli-1
0.1
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0

Fig. 18

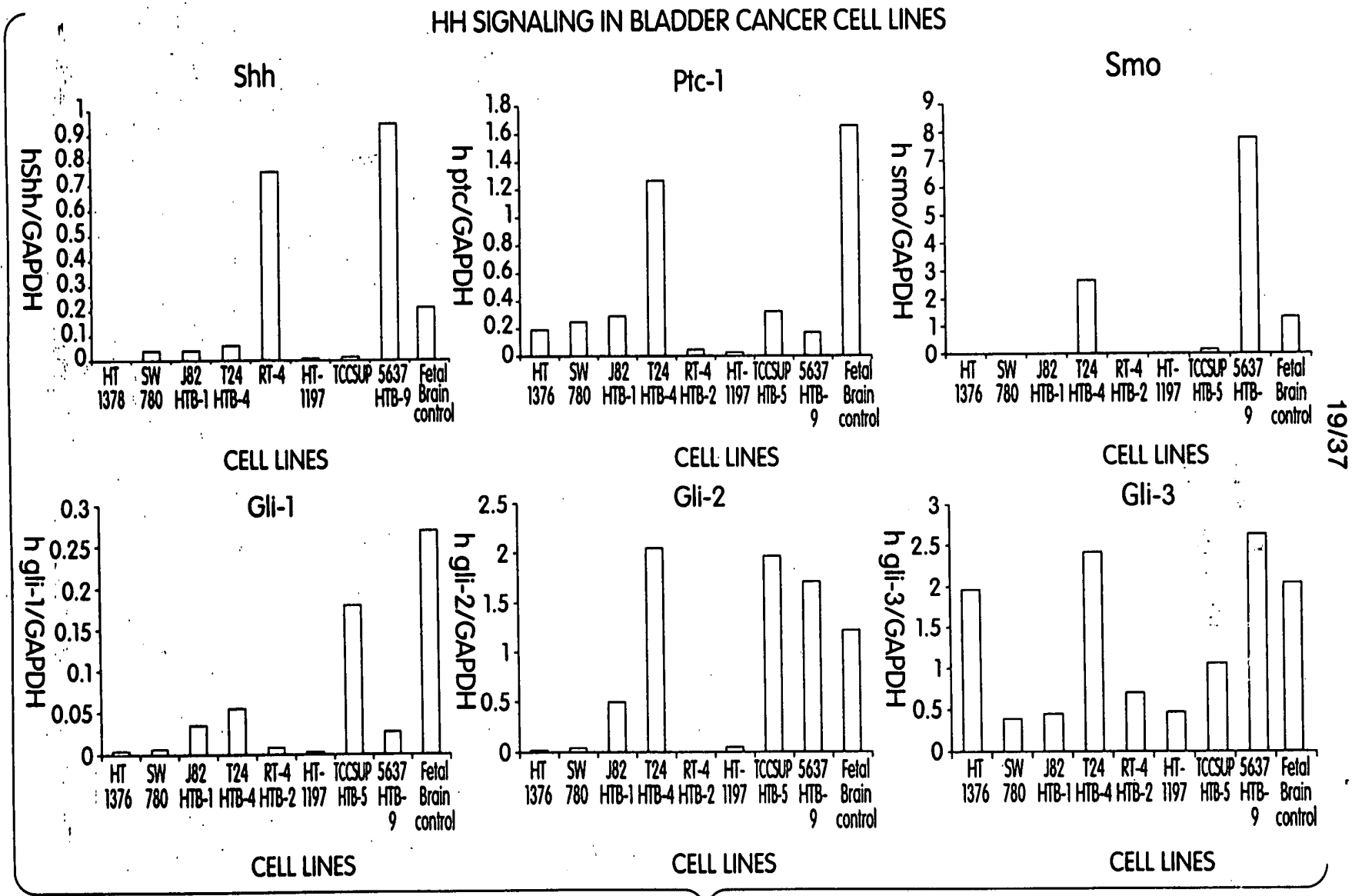
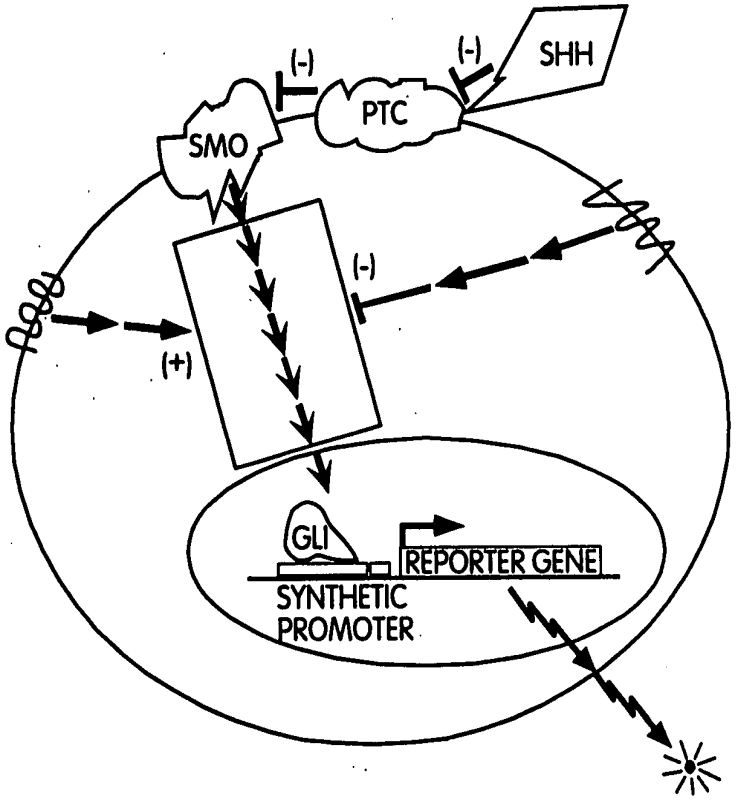

HH SIGNALING IN BLADDER CANCER CELL LINES
Shh
hShh/GAPDH
Ptc-1
h ptc/GAPDH
Smo
h smo/GAPDH
Gli-1
h gli-1/GAPDH
Gli-2
h gli-2/GAPDH
Gli-3
h gli-3/GAPDH
HT 1376
SW 780
J82 HTB-1
T24 HTB-4
RT-4 HTB-2
HT-1197
TCCSUP HTB-5
5637 HTB-9
Fetal Brain control
CELL LINES

Fig. 19

IN VITRO EFFICACY
Gli-luc ASSAY

S12 FIBROBLAST CELL LINE WITH LUCIFERASE REPORTER



B. Flow-chart

Day 1
Plate S12 + cancer cells
DMEM 10% FBS

↓

Day 2
Change to 1% FBS
Let grow 2d

↓

Day 4
Read luciferase activity on Topcount
or
Treat with cmpds (read next day)

Fig. 20

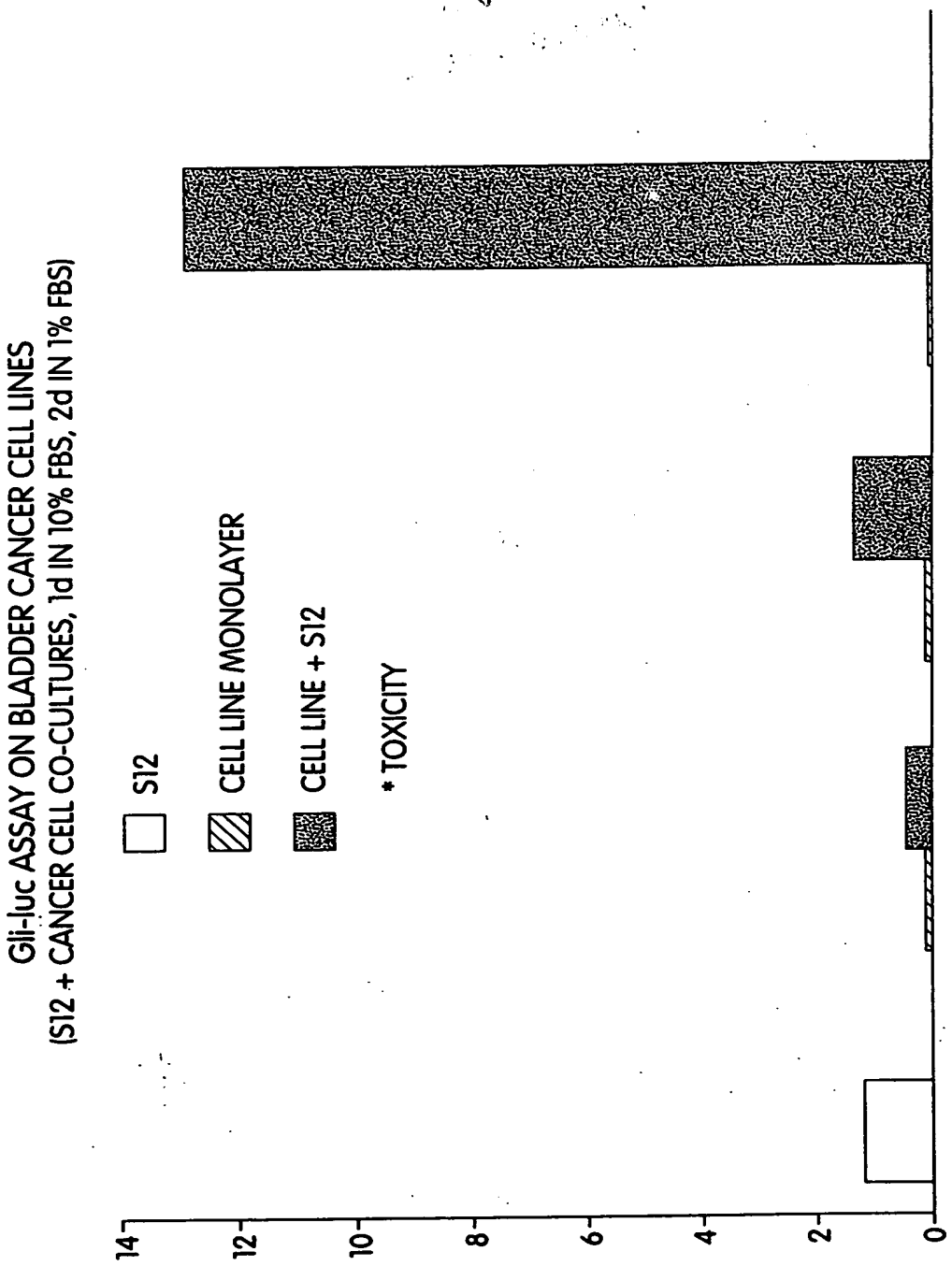


Fig. 21

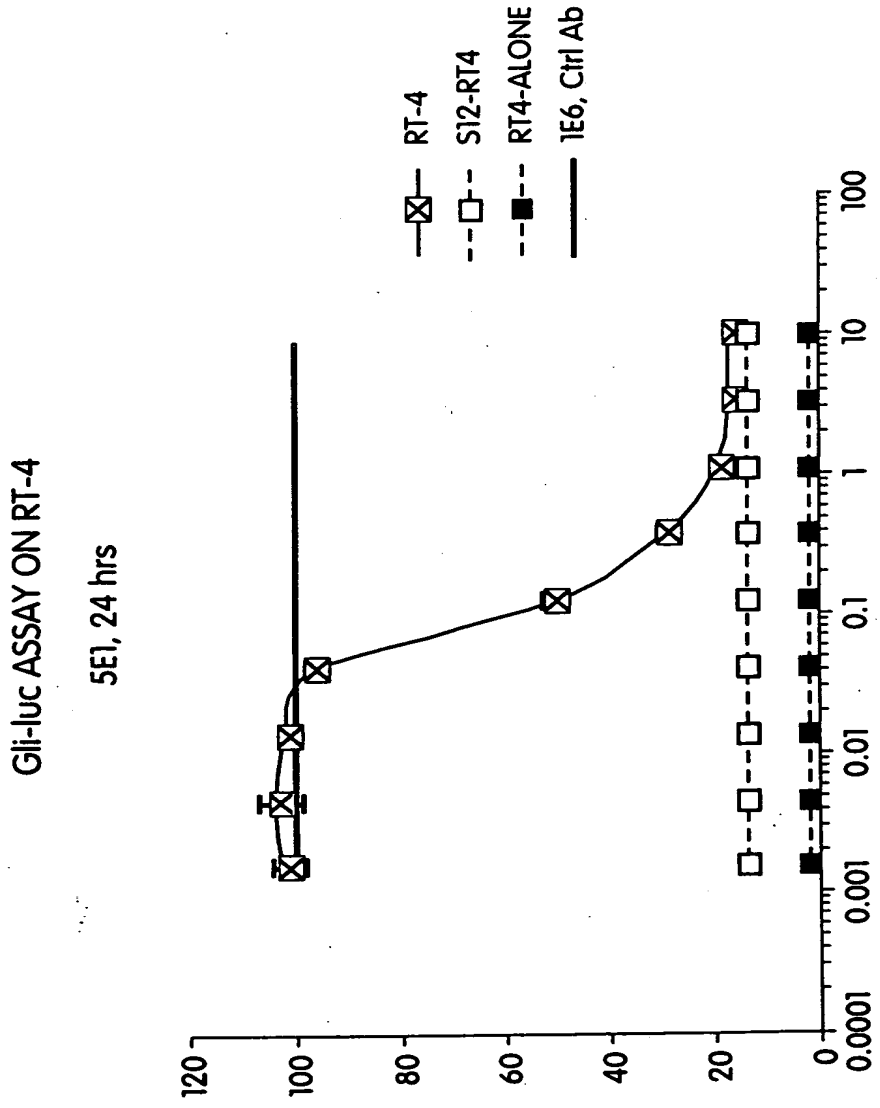

Gli-luc ASSAY ON RT-4
5E1, 24 hrs
RT-4
S12-RT4
RT4-ALONE
1E6, Ctrl Ab
120
100
80
60
40
20
0
0.0001
0.001
0.01
0.1
1
10
100
IC50 = 85ng/ml
IC90 = 500ng/ml

Fig. 22

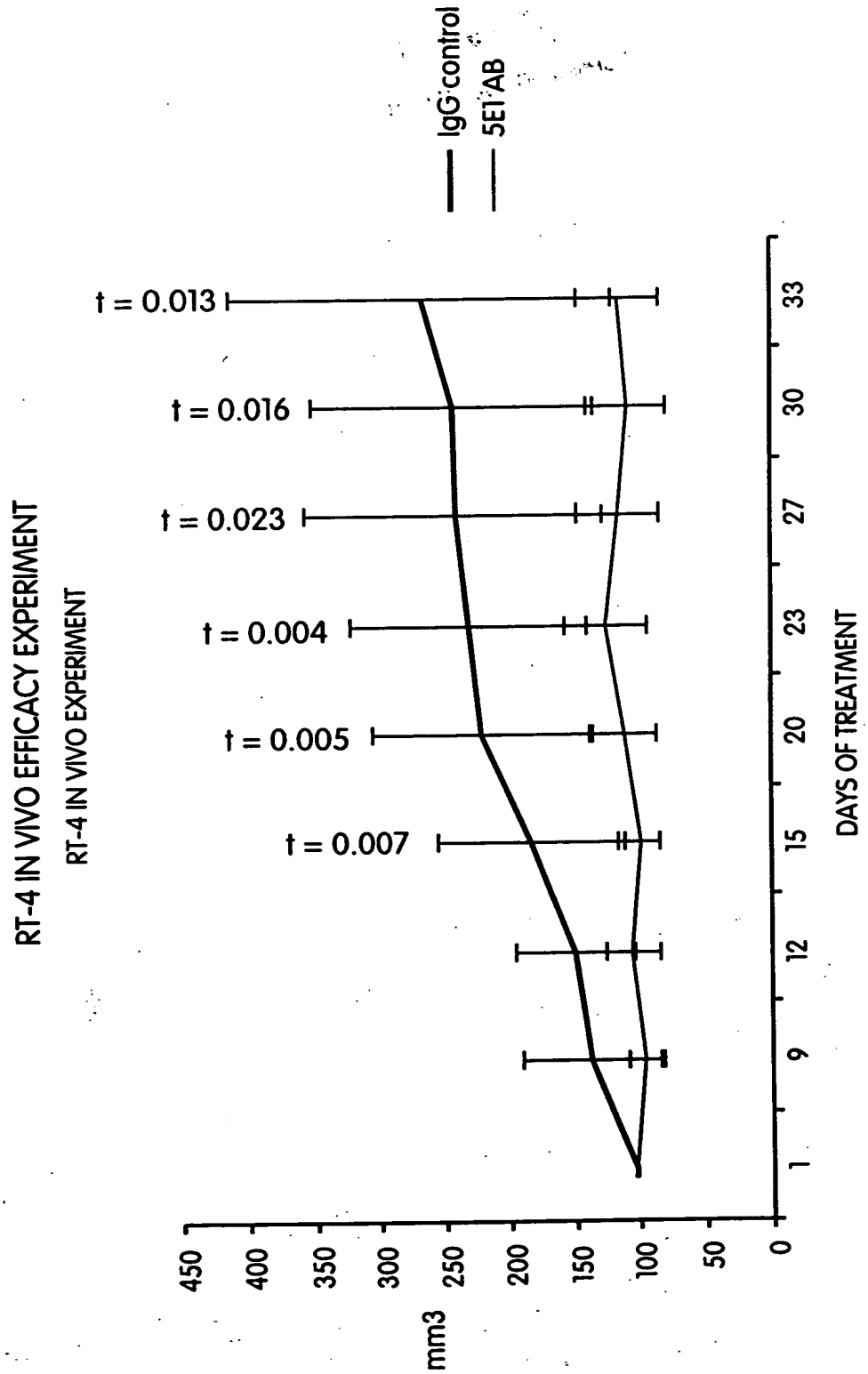

RT-4 IN VIVO EFFICACY EXPERIMENT
RT-4 IN VIVO EXPERIMENT
IgG control
5E1 AB
t = 0.013
t = 0.016
t = 0.023
t = 0.004
t = 0.005
t = 0.007
mm3
450
400
350
300
250
200
150
100
50
0
1
9
12
15
20
23
27
30
33
DAYS OF TREATMENT

Fig. 23

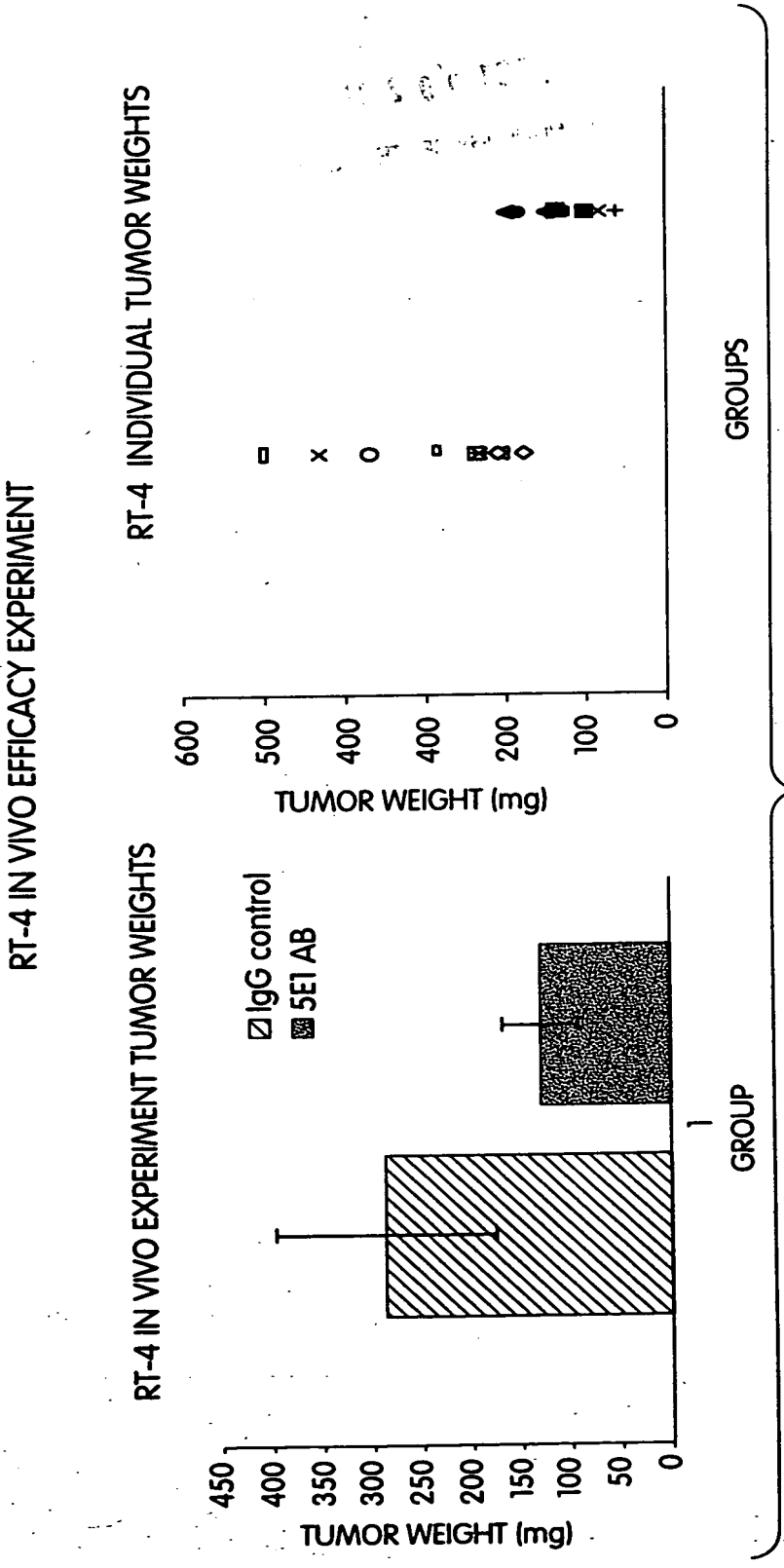

RT-4 IN VIVO EFFICACY EXPERIMENT
RT-4 IN VIVO EXPERIMENT TUMOR WEIGHTS
IgG control
5E1 AB
TUMOR WEIGHT (mg)
450
400
350
300
250
200
150
100
50
0
1
GROUP
RT-4 INDIVIDUAL TUMOR WEIGHTS
TUMOR WEIGHT (mg)
600
500
400
400
200
100
0
GROUPS

Fig. 24

MOUSE Gli-1 EXPRESSION IN 5E1-TREATED RT-4 TUMORS

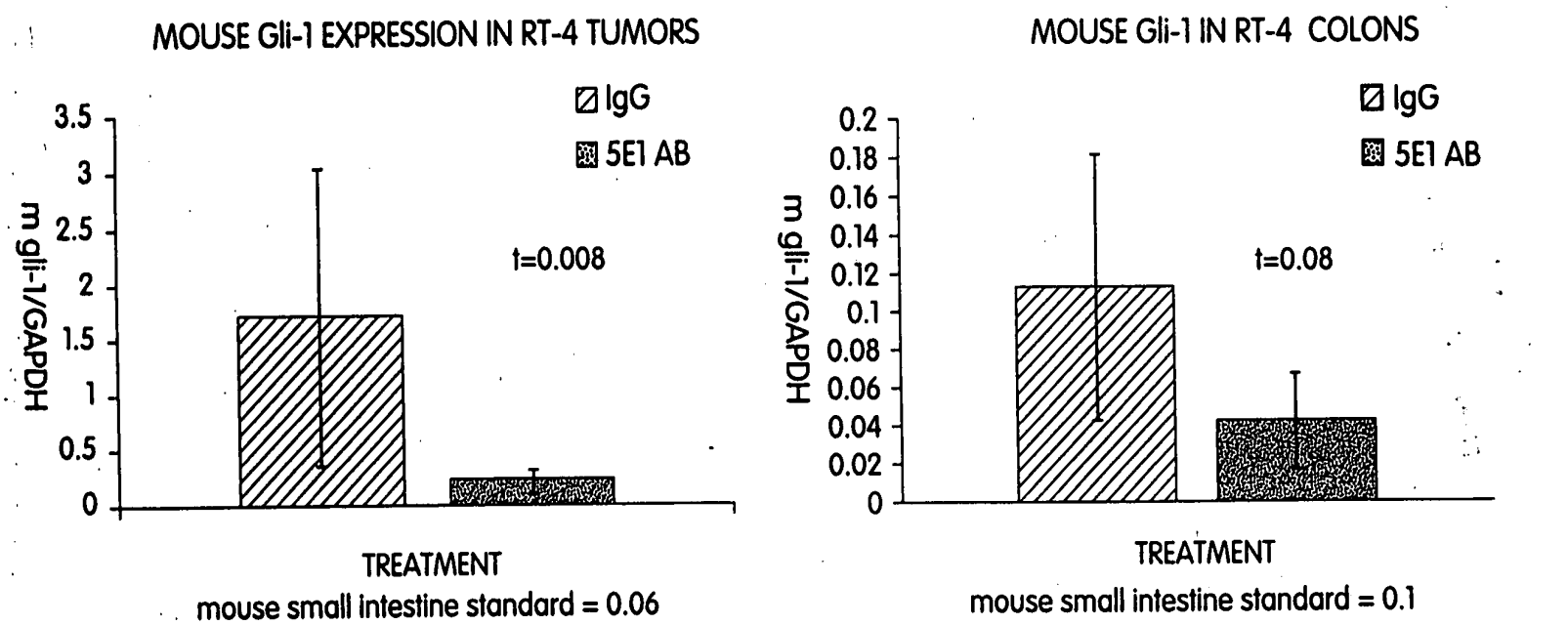


Fig. 25

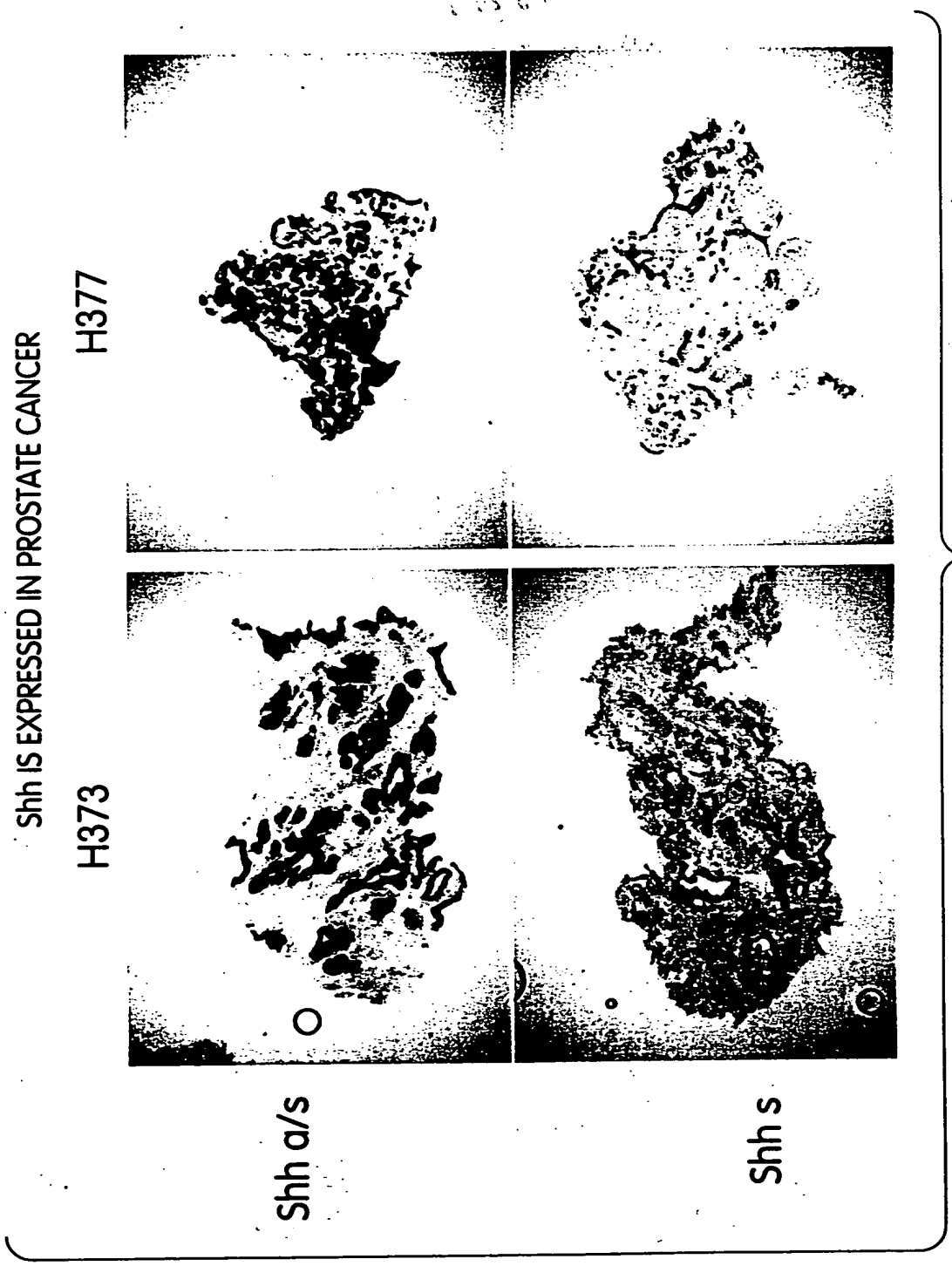


Fig. 26

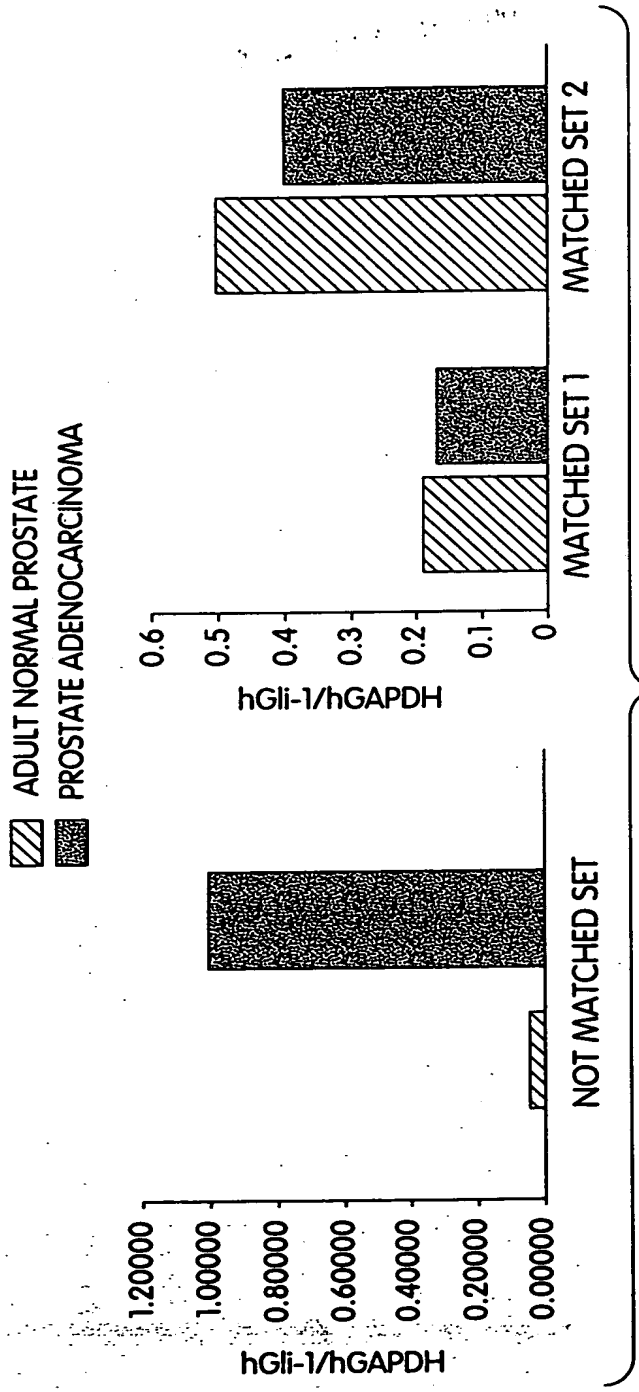

ADULT NORMAL PROSTATE
PROSTATE ADENOCARCINOMA
1.20000
1.00000
0.80000
0.60000
0.40000
0.20000
0.00000
hGli-1/hGAPDH
NOT MATCHED SET
0.6
0.5
0.4
0.3
0.2
0.1
0
hGli-1/hGAPDH
MATCHED SET 1
MATCHED SET 2

Fig. 27

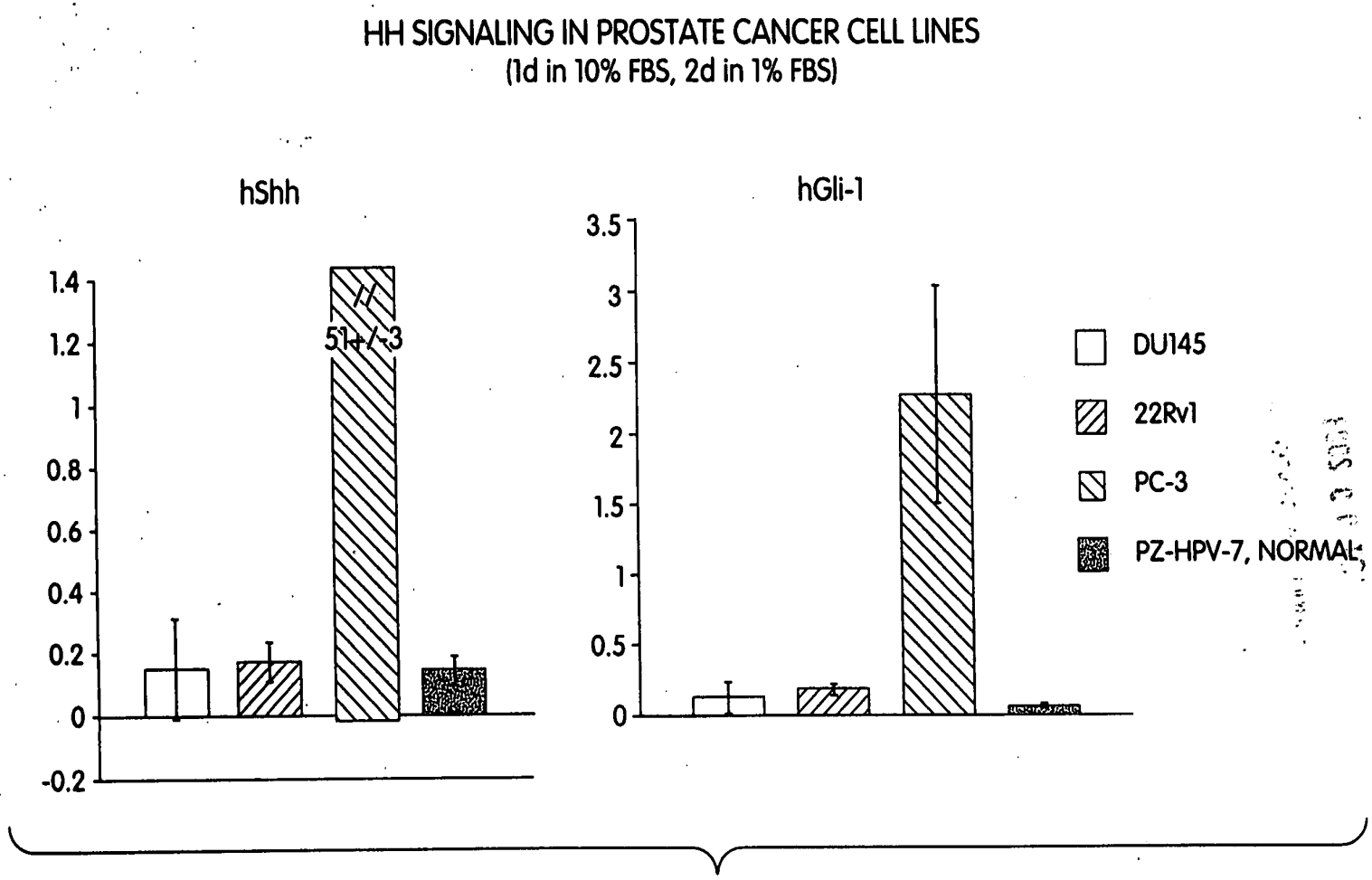

HH SIGNALING IN PROSTATE CANCER CELL LINES
(1d in 10% FBS, 2d in 1% FBS)
hShh
hGli-1
1.4
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
51+/-3
3.5
3
2.5
2
1.5
1
0.5
0
DU145
22Rv1
PC-3
PZ-HPV-7, NORMAL

Fig. 28

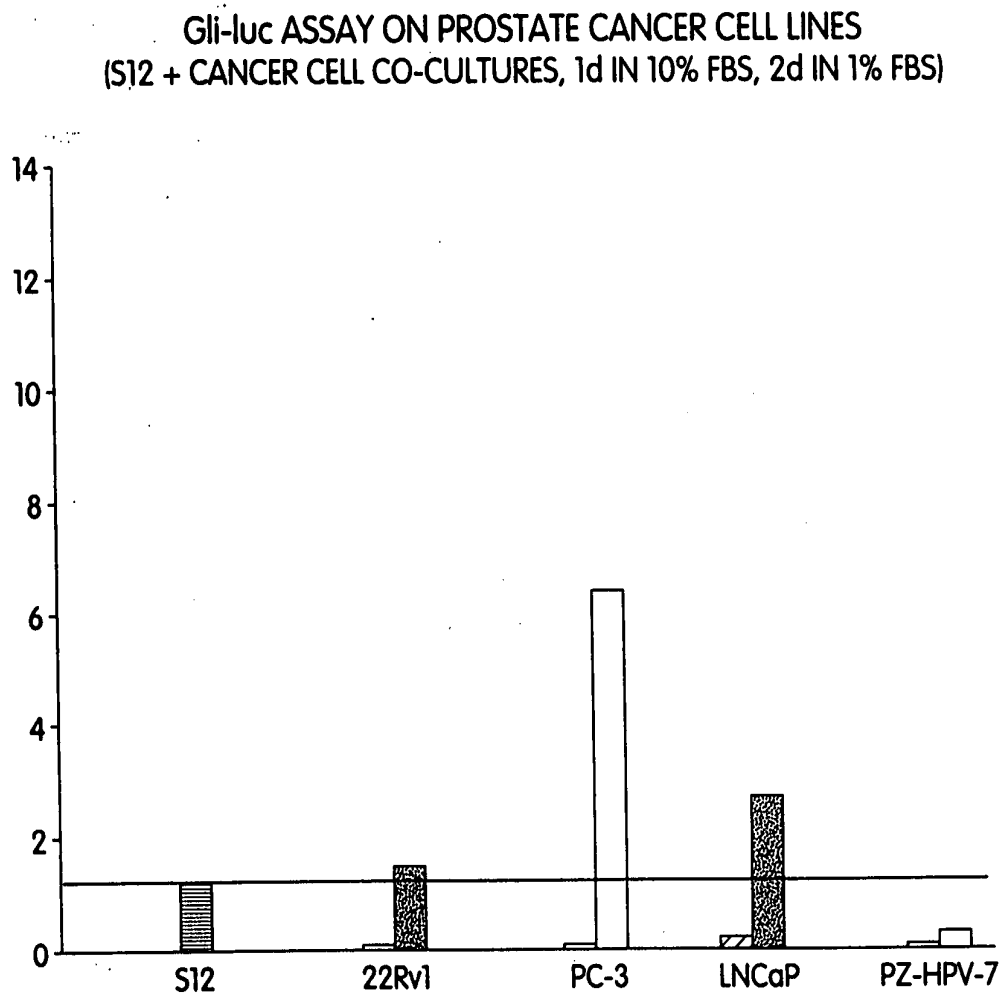

Gli-luc ASSAY ON PROSTATE CANCER CELL LINES
(S12 + CANCER CELL CO-CULTURES, 1d IN 10% FBS, 2d IN 1% FBS)
14
12
10
8
6
4
2
0
S12
22Rv1
PC-3
LNCaP
PZ-HPV-7

Fig. 29

IN VITRO EFFICACY
INHIBITION OF HEDGEHOG SIGNALING BY HEDGEHOG ANTAGONISTS
(Gli-luc, 24 hrs)

5E1

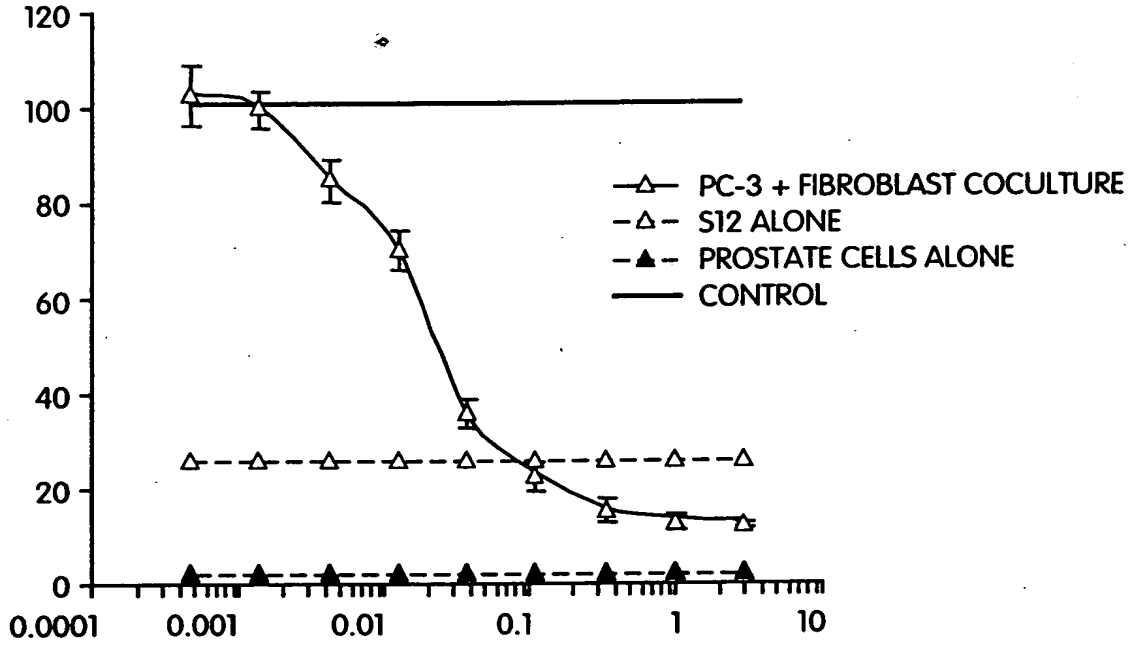


Fig. 30

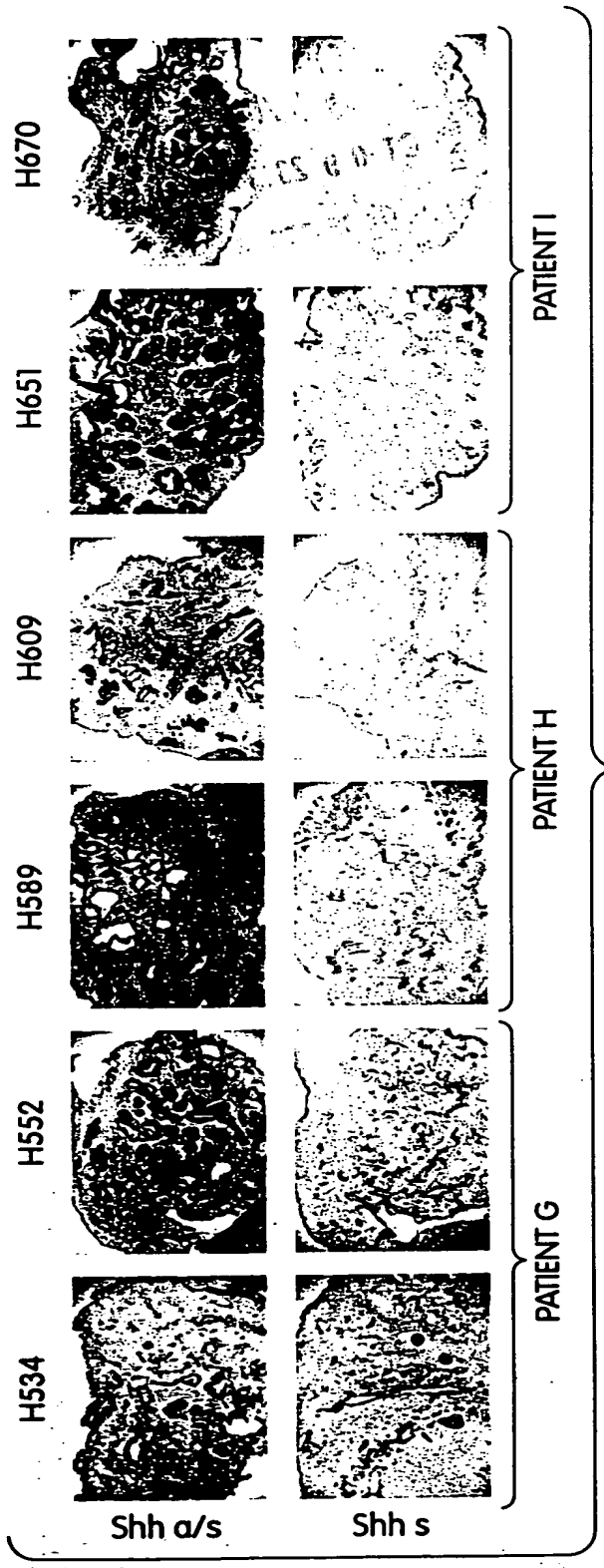


Fig. 31

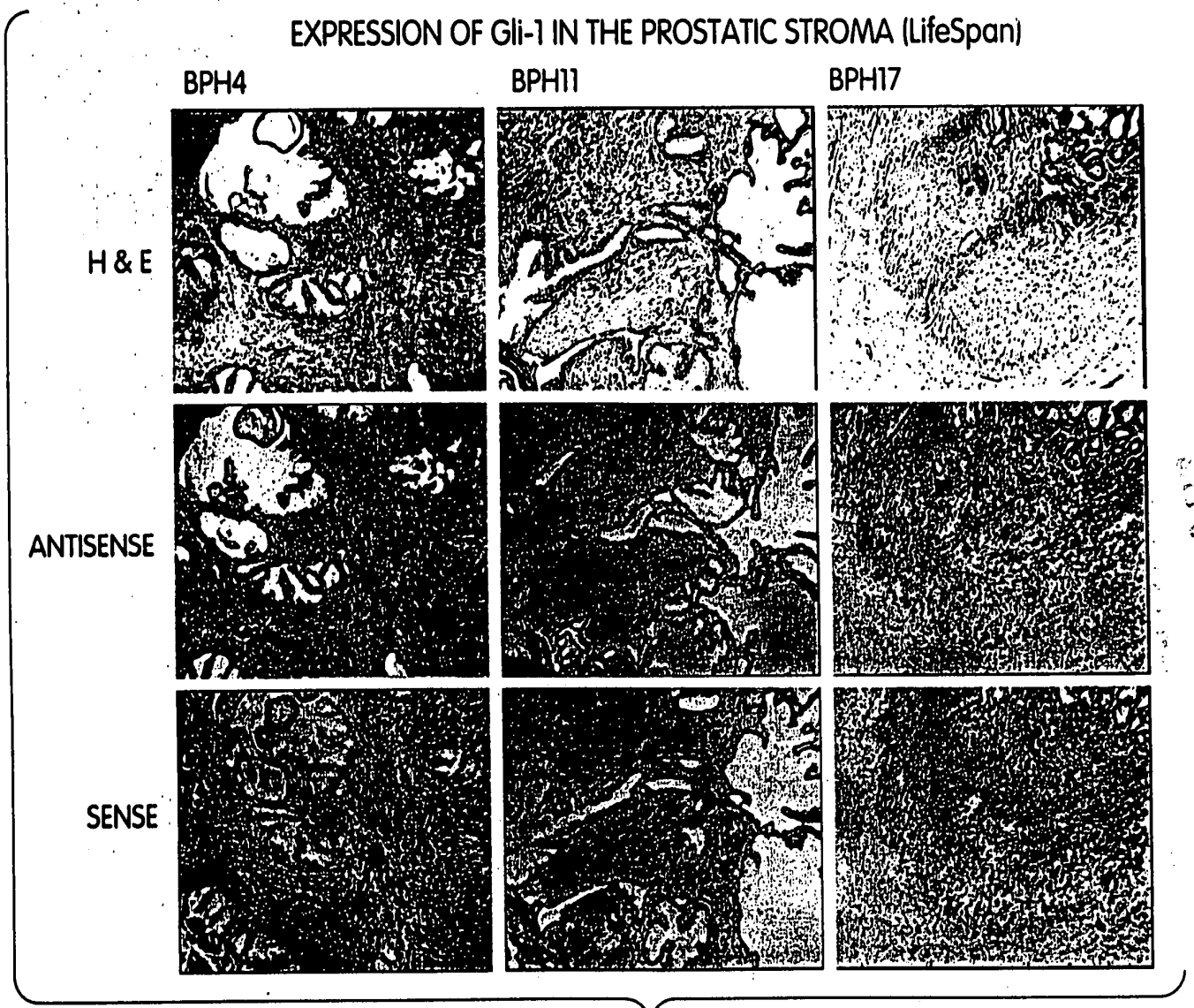


Fig. 32

PROXIMO-DISTAL Shh GRADIENT IN NORMALPROSTATE

Fig. 33

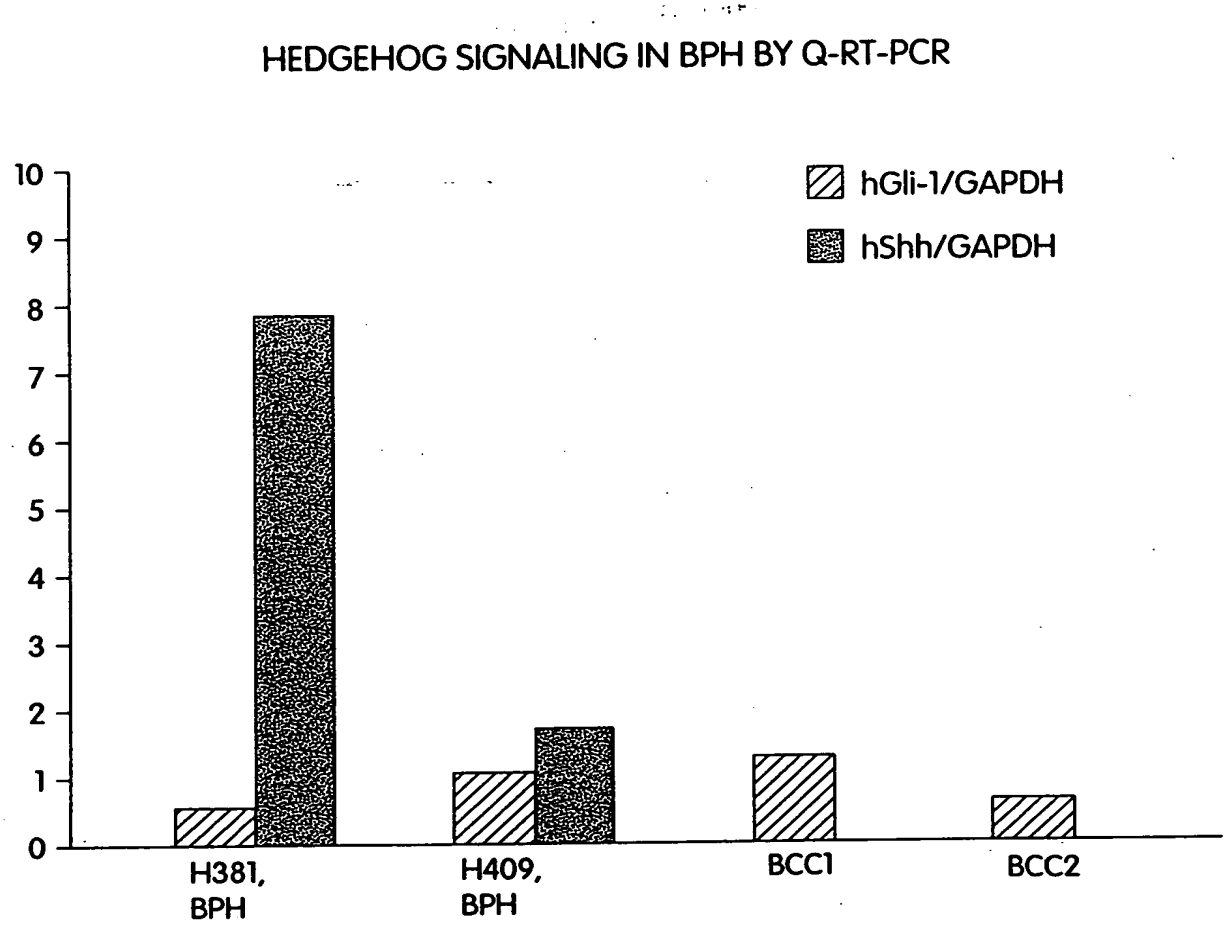

HEDGEHOG SIGNALING IN BPH BY Q-RT-PCR
hGli-1/GAPDH
hShh/GAPDH
10
9
8
7
6
5
4
3
2
1
0
H381, BPH
H409, BPH
BCC1
BCC2

Fig. 34

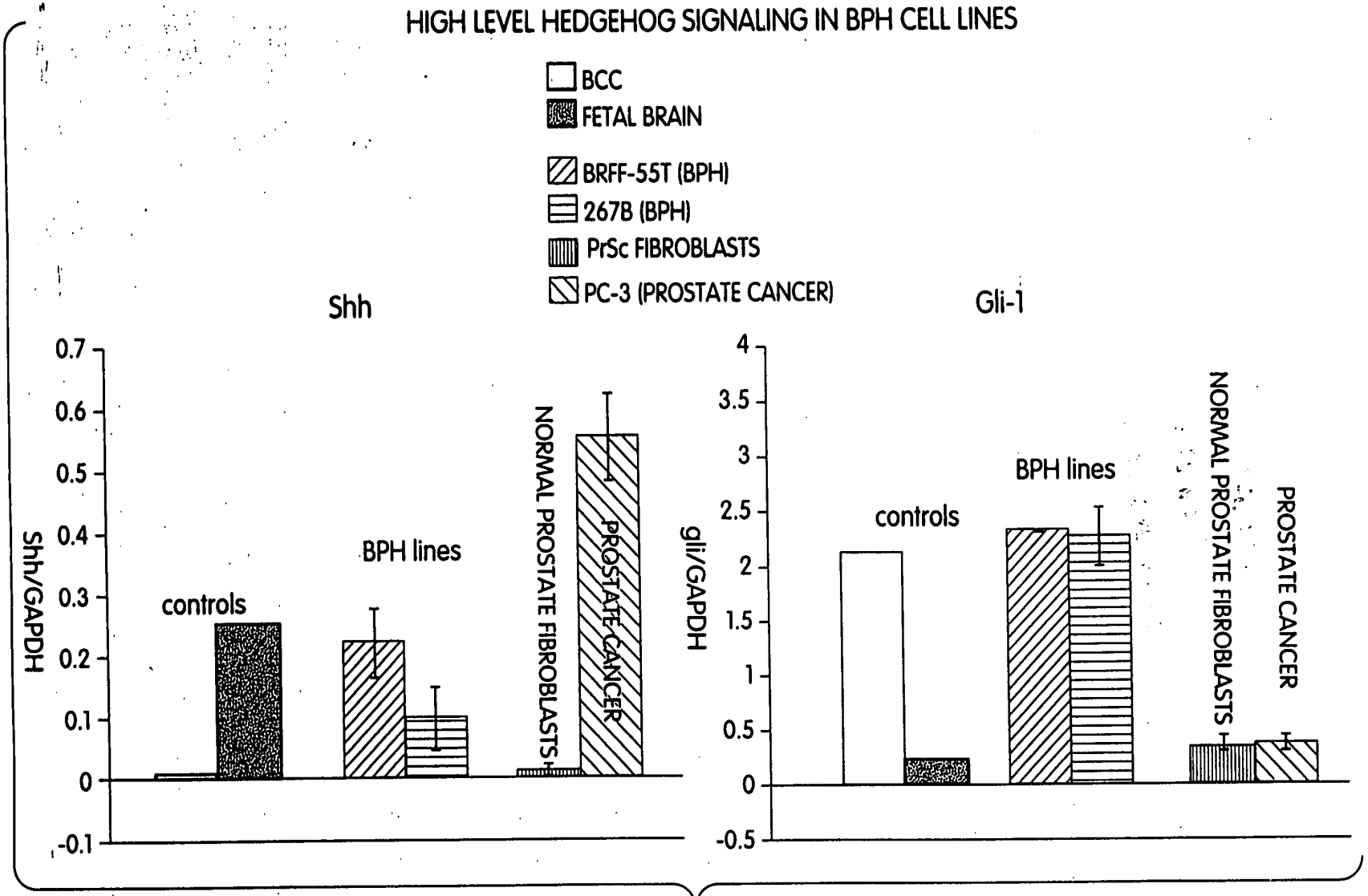

HIGH LEVEL HEDGEHOG SIGNALING IN BPH CELL LINES
BCC
FETAL BRAIN
BRFF-55T (BPH)
267B (BPH)
PrSc FIBROBLASTS
PC-3 (PROSTATE CANCER)
Shh
Shh/GAPDH
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
controls
BPH lines
NORMAL PROSTATE FIBROBLASTS
PROSTATE CANCER
Gli-1
gli/GAPDH
4
3.5
3
2.5
2
1.5
1
0.5
0
-0.5
controls
BPH lines
NORMAL PROSTATE FIBROBLASTS
PROSTATE CANCER

Fig. 35

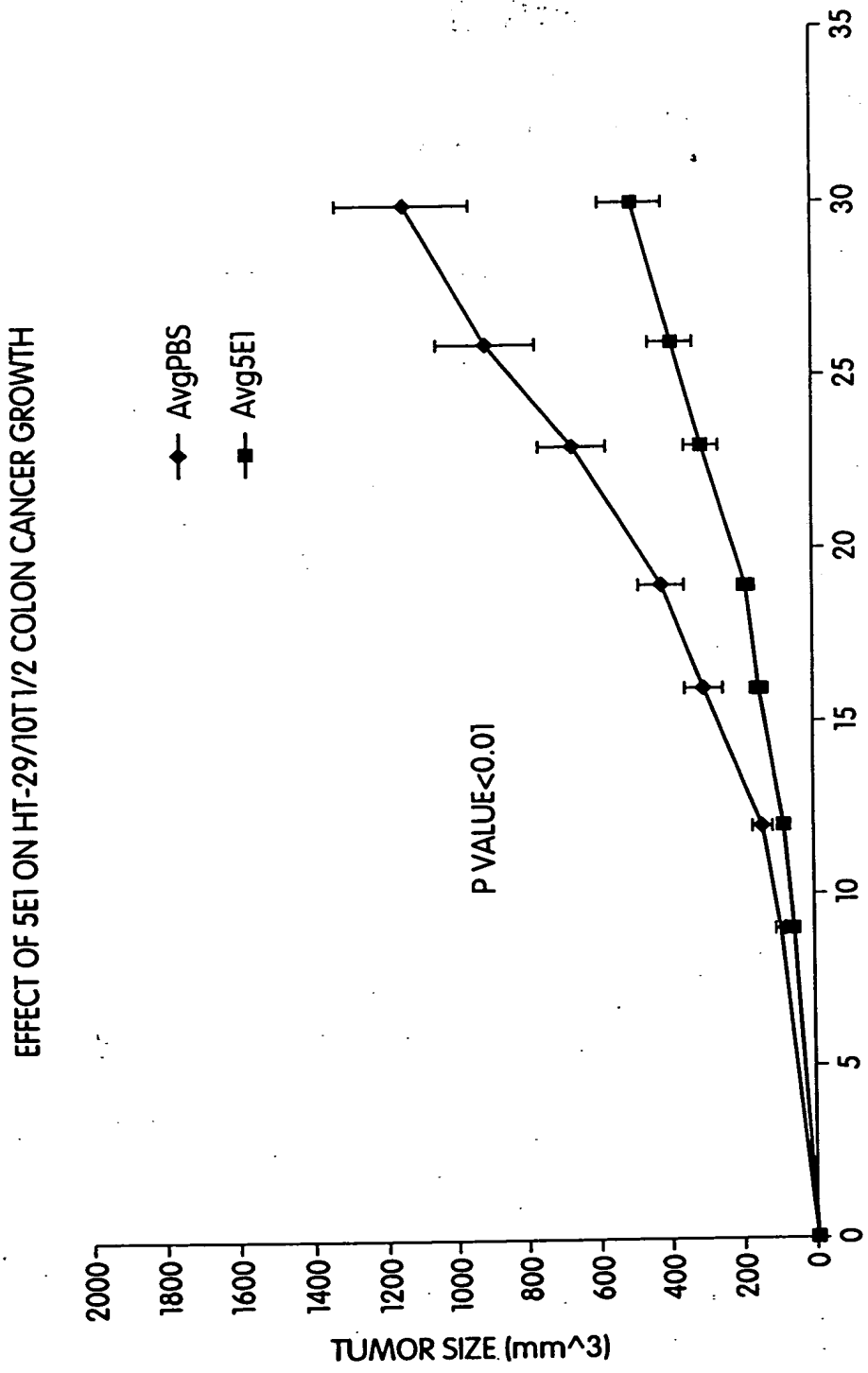

EFFECT OF 5E1 ON HT-29/10T 1/2 COLON CANCER GROWTH
AvgPBS
Avg5E1
P VALUE<0.01
TUMOR SIZE (mm^3)
DAYS
0
200
400
600
800
1000
1200
1400
1600
1800
2000
0
5
10
15
20
25
30
35

Fig. 36

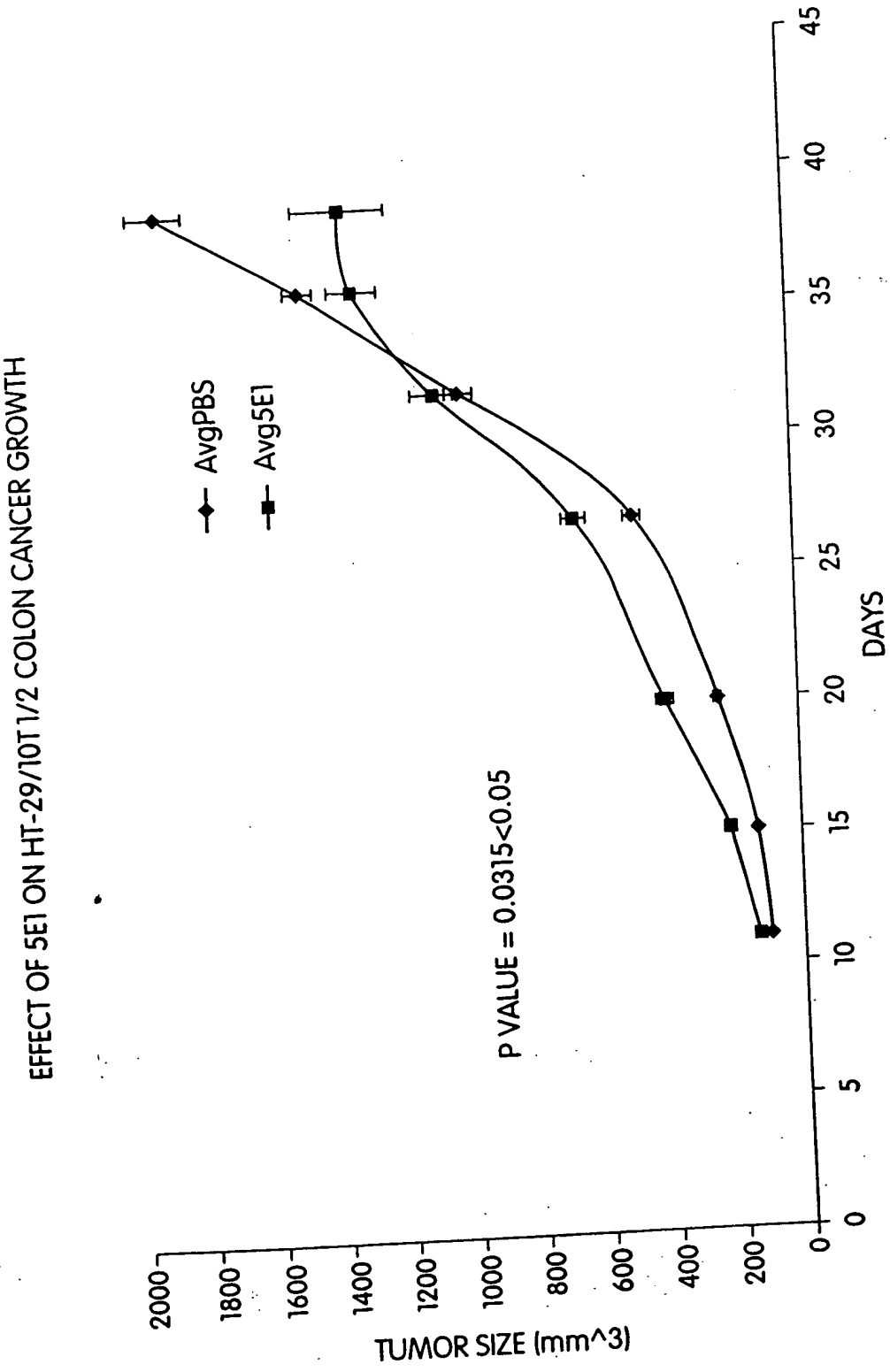


Fig. 37